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SUMARIO

ÓRGANO OFICIAL DE LA

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Organo oficial de la Asociación Latinoamericana de Ciencias Fisiológicas

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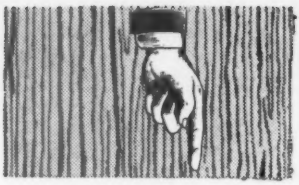

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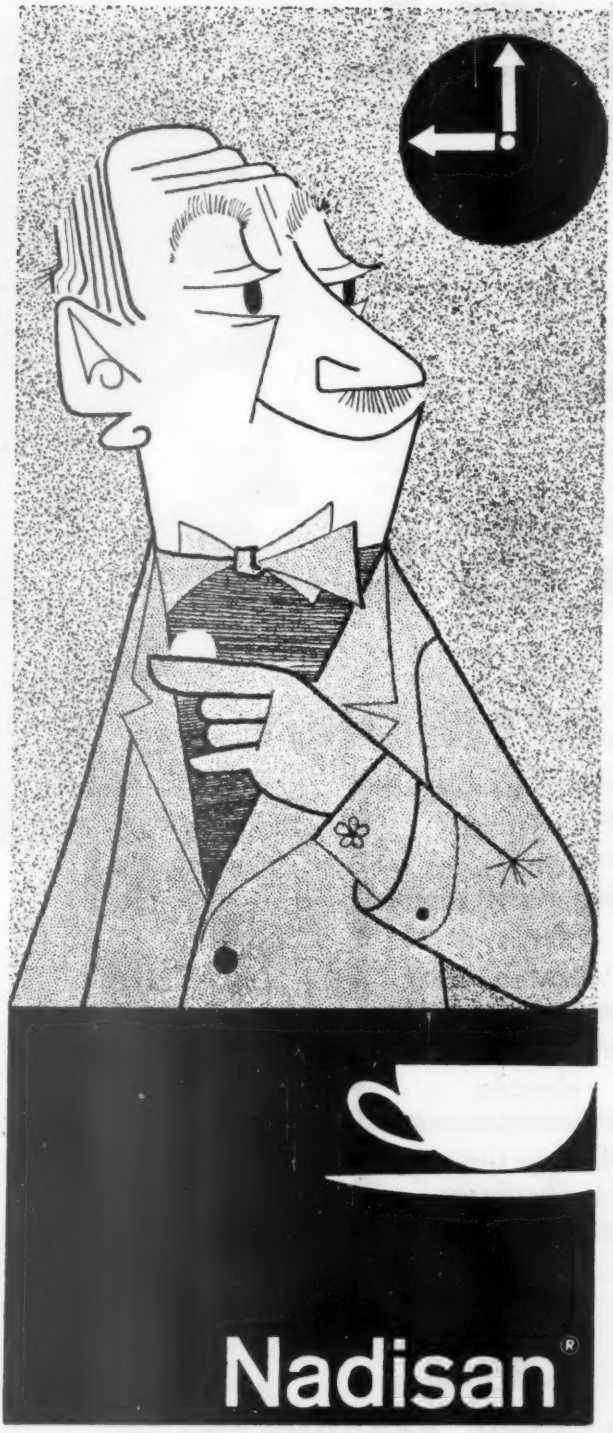

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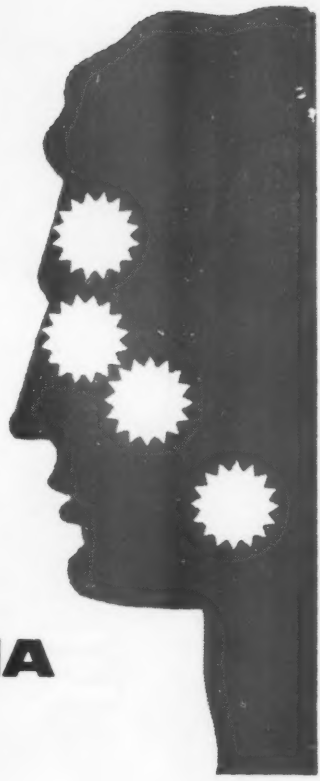

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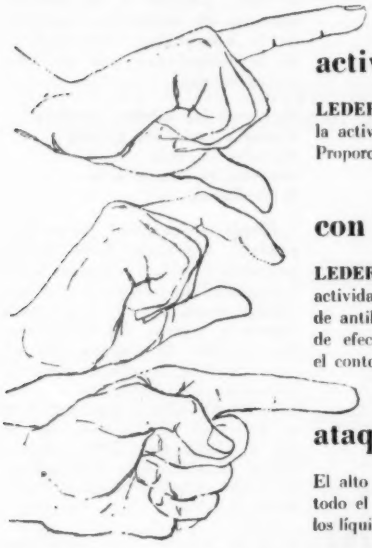

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empiece despacio

vaya despacio

La norma de "¡empiece despacio! ¡vaya despacio!" en el uso de **DBI**, permite a un número máximo de diabéticos disfrutar de la comodidad, bienestar fíde la terapéutica por vía oral en casos de:

diabetes del adulto, estable
diabetes inestable (labil)
diabetes juvenil
diabetes resistente a la sulfonilurea

"¡**Empiece despacio! ¡vaya despacio!**" Significa dosis inicial pequeña (25 ó 50 mg en dosis fraccionadas, por día) con aumenos pequeños (25 mg) cada tercer o cuarto día hasta que los niveles de azúcar sanguíneos quedan regulados adecuadamente. La inyección de insulina se reduce gradualmente al par que se aumenta la dosis de **DBI**. Con **DBI** solo se logra generalmente dominio satisfactorio de la diabetes estable ligera.*

Con la norma "¡**empiece despacio! ¡vaya despacio!**

Más de 3000 diabéticos sometidos a tratamiento diario con **DBI** se han estudiado cuidadosamente por períodos varios hasta de tres años. No se notaron cambios histológicos ni funcionales en el hígado, sangre, riñones, corazón ni en otros órganos.

DBI (N^1-β-fenetilbiguanida) se ofrece en tabletas blancas, ranuradas, de 25 mg cada una, en frascos de 30 y 100.

* Nuestros representantes en cada país enviarán, asolicitud, un libreto con instrucciones completas sobre la dosis para cada clase de diabetes y demás información pertinente.

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A CONDITIONED REFLEX WHICH REPRODUCES THE HYPOGLYCEMIC EFFECT OF INSULIN

R. Álvarez-Buylla and J. Carrasco-Zanini
(Laboratory of Physiology, Instituto Nacional de Neumología, "Gea González", Tlalpan, México, D. F.)

Many publications deal with the problem of the homeostasis of glucose. The role and mechanism of action of insulin in this homeostasis has been studied extensively. In spite of this, De Witt Stetten (1957) has recently reached the following conclusion: "the mechanism of insulin action is today, as far as I am aware, completely unknown".

The present knowledge of the role of the nervous system in the regulation of this homeostatic mechanism is still very limited.

With the use of the method of the conditioned reflexes of Pavlov, the present study was performed, in order to gain knowledge about the role of the nervous system in the neuro-humoral integration of glucose homeostasis.

Methods

The experiments were carried out in dogs in a chamber for the study of conditioned reflexes described in detail in a previous publication (Álvarez-Buylla, 1950). The animals used were mongrel dogs of both sexes and of different weights. The injections of insulin (Lilly) or saline solution (NaCl 0.8 per cent) were controlled from outside the chamber, in order to avoid other stimuli at the time of the injections. The intravenous route was chosen for the injections, using a poly-ethylene tube, previously inserted into one of the hind-leg veins of the animals, under local anesthesia. This tube was used also to obtain the necessary blood samples for the determination of glucose concentration. In order to avoid the formation of thrombi, the tube was filled with a solution of heparin (Hoffman La Roche) when not in use.

The glucose concentrations in the blood and urine were measured with the Folin-Wu photocolorimetric method. At the end of every experiment each dog was given 500 cc of saturated sucrose solution for the treatment of the hypoglycemia.

A number of preliminary tests were made to determine the correlation between the dose of insulin injected and the degree of hypoglycemia obtained, in order to establish an adequate dose of insulin for the experiments.

Received for publication, June 11, 1960.

There was a wide variation in the effect on the blood sugar of any given dose of insulin. It was not possible to outline with precision, the temporal course of the blood sugar changes. The dose of insulin was always the same for each animal, but varied for different dogs between 1 and 50 units per kg of body weight. There was also a wide variation in the initial blood glucose concentrations. The magnitude of the hypoglycemic response was related to the initial blood glucose concentration: greater reductions were associated with higher initial values. The correlation coefficient between the initial value and the reduction in blood glucose concentration, was significant, ($r = 0.613$; P 0.001). This correlation disappears when the fall in blood sugar is expressed as a fraction of the initial values ($r = -0.290$; P 0.05).

The electrocardiograms (EKG) were recorded using standard lead II. A pneumograph placed around the thoraco-abdominal region served for recording the respiratory movements. The injection of massive doses of insulin produced, in addition to hypoglycemia, tachycardia, inversion of the T wave of the EKG, tachypnoea, salivation, vomiting, sphincter release, and muscular twitches, which first were clonic and later became tonic-clonic. On some occassions there appeared spastic muscular contractions. At the final stage the dog entered into a state of coma, appearing inert and completely unaware of its surroundings. No correlation could be observed between the blood sugar levels and the magnitude of the other reactions which occurred during the hypoglycemic shock.

The pancreatectomies were performed aseptically under sodium pentobarbital (Abbott) anesthesia. The dogs were nursed for three or four days after the operation.

Before attempting to establish any conditioned reflexes, preliminary controls were performed on each dog to ascertain that the stimulus to be conditioned, sound of a bell, metronome, etc., did not produce any changes in the concentration of blood sugar. The special details of the method employed in the different experimental series will be described together with the corresponding results.

Results

A) *The hypoglycemic conditioned reflex in normal dogs.*

In a group of seven dogs which received as the inconditional stimulus 1 unit of insulin per kg of body weight, the conditioned reflex could be established. If the dose of insulin was large (50 units per kg) most of the dogs, 7 out of 8, died before the conditioned reflex could be established.

The experiments were performed according to the following plan. On the first day, the dog was taken to the chamber for conditioning reflexes and kept on the table for one hour. On the second day the same procedure was repeated but, in addition, the EKG electrodes and pneumograph were attached to the animal and the plastic tube previously introduced into the vein was connected to the injection system. On the third day, after repeating the same procedure, 5 ml of saline solution were injected intravenously and at the same time the stimulus to be conditioned (metronome, bell or light) was turned on.

Once it was made certain that this procedure did not alter any of the chosen indicators, the process of conditioning was begun on the fourth day, as follows. An initial blood sample was taken. During 5 minutes the basal and respiratory rates were recorded. Insulin was then injected and the stimulus to be conditioned was turned on for 15 minutes. At the end of this period the conditioned stimulus was termina-

FE DE ERRATA

Nº 3, vol. X, 1960, page 155

FIG. 1: it reads: "*Black symbols*: values obtained when the conditioned stimulus was applied without insulin injection".

It should read: "*Black symbols*: values obtained when insulin was injected – *White symbols*: values obtained when the conditioned stimulus was applied without insulin injection".

Nº 3, vol. X, 1960, page 167 6th line

Left, it reads: "ministration (Table II, Fig. 1). At this".

It should read: "plasma (Table V). After reaching its".

ted and a second blood sample was taken. After repeating this maneuvre, usually during 8 consecutive days the same procedure was performed again, except that, instead of insulin, an equal volume of saline solution was given (sham injection). The glucose concentration in the blood decreased to the same extent as had occurred on the eight previous days after insulin injection. In other words, the hypoglycemic action of insulin was reproduced by a conditioned stimulus.

The results obtained were similar in all seven dogs. The basal blood glucose concentration had a mean value of 92 mg per 100 ml of blood. The intravenous injection of insulin (1 mg per kg of body weight) lowered the blood glucose concentration after 15 minutes to a mean value of 46.5 mg per 100 ml of blood.

On the days on which insulin was substituted by a sham injection, the basal blood glucose concentration had a mean value of 90.5 mg per 100 ml of blood. The conditioned stimulus produced a hypoglycemia which after 15 minutes had a mean value of 52 mg per 100 ml of blood. Typical results obtained in one of the dogs are shown in figure 1.

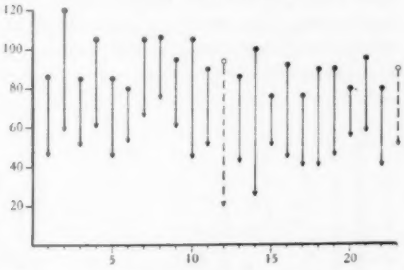

FIG. 1. — *Conditioned hypoglycemic reflex in the normal dog.* Abscissae: *ordinal numbers of the experiments performed at daily intervals.* Ordinates: *blood glucose concentration in milligrams per 100 ml of blood.* Circles: *blood glucose concentration at the beginning of each experiment.* Triangles: *blood glucose concentration fifteen minutes after the injection of insulin or the application of the conditioned stimulus.* Vertical lines: *change of blood glucose concentration in each experiment.* Black symbols: *values obtained when the conditioned stimulus was applied without insulin injection.*

The hypoglycemic conditioned reflex occurred as a consequence of an auditory stimulus applied together with the intravenous injection of saline solution. It was interesting to determine whether the injection of saline solution or the auditory stimulus or both were responsible for initiating the conditioned reflex which produces hypoglycemia. After having established the reflex in 3 dogs, the effect of the saline injection alone, and the action of the auditory stimulus alone were tested. The injection of saline solution was without any appreciable effect, while the auditory stimulus produced a hypoglycemia which appeared as great as that produced by the combination of both factors.

Once the conditioned reflex was established, it was interesting to learn about its stability by determining the temporal course of its extinction. These determinations were made on three animals. In two dogs the doses of insulin which had been used as an unconditioned stimulus was 1 unit per kg of body weight; in the third, the dose of insulin was 50 units per kg of body weight. This was the only animal among 8, which received this high dose of insulin and survived a sufficiently long period to enable it to acquire the conditioned reflex. In this dog after associating the injection of insulin with the sound of the metronome for 8 consecutive days the same procedure was repeated on the following days, except that insulin was substituted by the sham injection. The conditioned hypoglycemia diminished progressively, due to lack of reinforcement (insulin injection) until, on the fifth day, the conditioned reflex was extinguished,

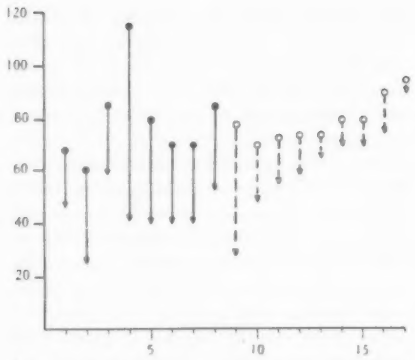

FIG. 2.—*Extinction process of the conditioned hypoglycemic reflex. Abscissae, ordinates, and symbols as in figure 1.*

that is, the dog did not lower its blood glucose concentration anymore with the sound of the metronome. Fig. 2 illustrates this experiment and shows the time course of the extinction process.

B) *Absence of glycosuria.*

One possible mechanism for the conditioned reduction in the blood glucose concentration could be the excretion of glucose through the urine. If this was its mechanism, the conditioned hypoglycemia should always be accompanied by glycosuria. In order to test this hypothesis, all experiments included the determination of glucose in the urine. There was no appreciable quantity of glucose in the urine, in all instances.

C) *The role of the pancreas.*

Another possible mechanism for the conditioned hypoglycemia could be a reflex stimulation of the beta cells of the Langerhans islets, whereby insulin would be secreted. This reflex stimulation might be susceptible to conditioning. To test this hypothesis two series of experiments were undertaken.

Conditioned hypoglycemia in the alloxan diabetic dog:

Two dogs were injected with a single dose of alloxan. For two and a half months they had a sustained hyperglycemia ranging between 220 and 330 mg per 100 ml of blood, in spite of insulin treatment (1 unit per kg and per day) and of a diet poor in carbohydrates. The conditioned experiments on these dogs were similar to those already described. A metronome was used as the stimulus. Figure 3 illustrates the results in one of the animals. The 4 preliminary control experiments, performed at daily intervals, showed no changes in blood glucose concentrations. From the 5th to the 13th experiments the sound of the metronome was reinforced by the injection of insulin (1 unit per kg). From the 14th to the 16th day, insulin was replaced by the sham injection. The sound of the metronome reduced the blood sugar concentration by 233, 150 and 76 mg per cent, on the 14th, 15th and 16th days, respectively. The decreasing hypoglycemia responses indicate the beginning of the extinction process mentioned above.

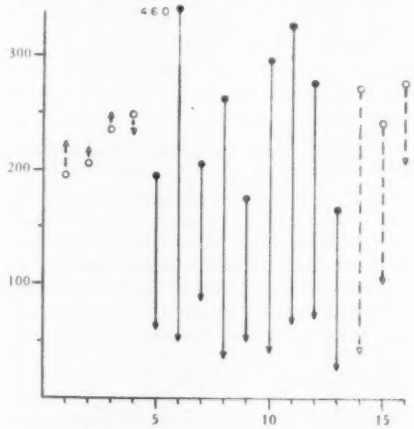

FIG. 3.—*Conditioned hypoglycemic reflex in the alloxan diabetic dog. Abscissae, ordinates, and symbols as in figure 1.*

These experiments suggest that the conditioned hypoglycemia is not due to an increased insulin production by the pancreas. The diabetes was so pronounced that it was unlikely that the Langerhans islets could produce, in response to the conditioned stimulus, the necessary amounts of insulin to reduce the blood sugar importantly. Nevertheless, this possibility could only be discarded if it could be proven that the alloxan had completely inactivated all of the beta cells, or by using completely depancreatized dogs.

Conditioned hypoglycemia in totally depancreatized dogs:

Twenty three dogs were used. Only in three cases it was possible to obtain animals which survived three weeks after pancreatectomy in sufficiently good conditions to try to establish the conditioned reflex. In these three dogs, the results were similar and it was possible to condition the reflex, so that the sound of the bell produced a conditioned hypoglycemia. Figure 4 illustrates the results in one of the animals. During nine consecutive days the sound of the bell was reinforced with the injection of insulin (1 unit per kg). On the 10th day, the conditioned stimulus was applied, but without the insulin reinforcement; it produced a reduction in blood glucose concentration of 109 mg per 100 ml. The sound of the bell was reinforced again on the 11th and 12th days with insulin. On the 13th day, the sound of the bell alone, reduced the blood glucose concentration by 136 mg per 100 ml.

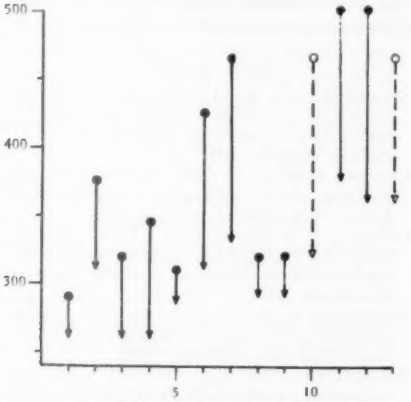

FIG. 4. — *Conditioned hypoglycemic reflex in the totally depancreatized dog. Abscissae, ordinates, and symbols as in figure 1.*

DISCUSSION

Savchenko (1939) reported the possibility of reproducing, by means of conditioned stimuli, the effects of several substances, among them the hypoglycemic effect of insulin. In 1954 Leites and Pavlov published a more extensive study, performed in a conditioned reflex chamber. These authors confirmed the earlier observations of Savchenko, and suggested that the cerebral cortex intervenes both in the production of the conditioned insulin hypoglycemia and in the development of the compensatory hyperglycemia.

Our observations, made on the alloxanized and the totally depancreatized dogs, exclude the possible role of insulin of pancreatic origin in the production of the conditioned hypoglycemia. Since, the responses obtained in these dogs (figs. 3 and 4) were as ample as those found in the normal dogs, the conclusion can be reached that the pancreas is not indispensable to produce the reflex hypoglycemia.

The fact that the hypoglycemic action of insulin can be conditioned, indicates that at least part of the insulin effect is mediated by a nerve reflex. Since there is no appreciable difference in the magnitude of the hypoglycemic responses to insulin and those to the conditioned stimulus it appears as though, under these conditions, insulin has only a slight if any peripheral action, and most of the effect is mediated by the reflex.

Since relatively small amounts of insulin (1 unit per kg) can be used for conditioning the hypoglycemic reflex (figs. 1, 3 and 4), it is likely that this reflex may participate in the normal regulation of blood sugar.

There is evidence (Gemmill, 1939) which shows, by in vitro experiments, that insulin acting peripherally is capable of increasing the uptake of glucose by tissues like the isolated rat diaphragm. This has been interpreted as due to the fact that insulin increases the cell permeability to glucose. This evidence does not invalidate the hypothesis that insulin may exert its physiological effect, in mammals, through the mediation of the central nervous system, at lower concentrations than are necessary to show its effects in vitro.

The pronounced hypoglycemic response to the conditioned stimulus, in the alloxan diabetic dog and in the dog chronically deprived of all its pancreas, indicates that there exists a potent factor, different from pancreatic insulin, but as effective as this hormone in lowering the blood glucose concentration. In other words, since the reduction in blood glucose concentration occurs without an exogenous, or any known endogenous supply of insulin, it appears necessary to postulate the existence of an unknown and potent hypoglycemic factor, which would be active during the hypoglycemic conditioned reflex. This factor would play an important physiological role in the hypoglycemic effect of insulin.

Summary

1) It was found that the hypoglycemic action of insulin can be conditioned in the dog.

2) The conditioning has been produced associating the hypoglycemic state, due to an insulin injection, with an indifferent auditory stimulus for eight consecutive days.

3) When the indifferent stimulus is applied alone on the ninth day, a hypoglycemic response appears, as large as that produced by insulin.

4) Similar conditioned hypoglycemias have been obtained in alloxan diabetic and in totally depancreatized dogs.

5) When the conditioned stimulus is applied repeatedly without reinforcement, the hypoglycemic response decreases progressively until it is extinguished.

6) The conditioned hypoglycemia is not due to a renal excretion of glucose or to a reflex stimulation of the beta cells of the Langerhans islets.

7) It is concluded that in the hypoglycemic action of insulin a nerve reflex is involved which can be conditioned. There appears to be a potent hypoglycemic factor, which is set in action by insulin through this nerve reflex.

Acknowledgment

The authors wish to express their indebtness to Drs. Arturo Rosenblueth and J. J. Mandoki for the careful reading and correction of the manuscript and to Eli Lilly & Co., Hoffman La Roche and Abbott Laboratories for the generous supply of the substances used.

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ARGINASA DE LA SANGRE Y DEL HÍGADO EN RATAS INTOXICADAS CON TETRACLORURO DE CARBONO

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La destrucción aguda de un tejido animal produce frecuentemente la penetración a los espacios vasculares de algunos constituyentes celulares dotados de propiedades bioquímicas específicas. Numerosas investigaciones han señalado la elevación de diversas actividades enzimáticas en la sangre, como consecuencia de procesos necróticos del hígado y del miocardio [2, 13, 19, 21, 22].

La administración de tetracloruro de carbono a los animales causa una lesión del hígado, que comienza en el centro del lobulillo y se extiende hacia la periferia. El desarrollo de esta lesión se caracteriza por anomalías morfológicas de las células, necrosis, infiltración y degeneración adiposa, y por alteraciones bioquímicas que se reflejan en la variación de la cantidad y reparto de algunos componentes celulares, en la modificación de numerosas actividades enzimáticas y en la pérdida de la función mitocondrial.

En los animales ureotélicos, la hidrólisis de la arginina por acción de la arginasa es una propiedad altamente específica de la célula hepática. La arginasa, abundante en el hígado, no existe en la sangre de varias especies examinadas, incluyendo la rata. Una excepción interesante es la especie humana, que posee esta enzima, en cantidad fácilmente demostrable, en el interior de los eritrocitos [4].

En este trabajo se demuestra que la destrucción del parénquima hepático en la rata por ingestión de tetracloruro de carbono, provoca la penetración de arginasa en el torrente sanguíneo. Como la sangre de la rata no intoxicada está prácticamente desprovista de arginasa, la aparición y el nivel de esta enzima en el plasma sanguíneo, constituyen una manifestación muy sensible de la extensión y evolución de la necrosis hepática causada por el tetracloruro de carbono.

Recibido para publicación, Junio 6, 1960.

Material y Métodos

Se produjo necrosis hepática en la rata por ingestión de una sola dosis de tetracloruro de carbono y se midieron las actividades arginásicas del plasma y del hígado durante las horas siguientes.

Intoxicación con CCl_4: Se utilizaron

ratas blancas Wistar, hembras y machos, que pesaban 200 ± 20 g. Después de un ayuno de 24 horas se les administró por intubación gástrica, bajo suave anestesia etérea, 0.2 ml de CCl_4 disuelto en 0.3 ml de aceite de oliva. Los animales se mantuvieron con su dieta habitual.

Se sacrificaron grupos de 4 a 6 ratas, a las 6, 12, 24, 36, 48, 72, 96, 120 y 162 horas después de la ingestión del tóxico y se determinó en ellas la actividad arginásica del plasma sanguíneo y del hígado, aplicando la reacción colorimétrica de Archibald [1]. Se tomaron muestras del órgano para examen histológico, fijándolas en formalina al 10 %.

Como testigos se usaron ratas no intoxicadas y ratas a las cuales se les administró oralmente una dosis única de 0.3 ml de aceite de oliva.

Determinación de la arginasa.

a) *En el plasma.* Se recogen 5 ml de sangre carotídea sobre 10 mg de oxalatos de potasio y amonio. El plasma se aisla por centrifugación.

Como etapa previa, la arginasa del plasma se activa, incubando a 37°C durante 1 hora, 0.5 ml de plasma no diluído o diluído en razones de 1 : 5 a 1 : 100 en solución isotónica de ClNa, con 1 ml de SO_4Mn 0.004 M.

La actividad de la enzima se mide agregando a la mezcla 1 ml de clorhidrato de arginina 0.1 M, disuelto en amortiguador 0.1 M de glicina, pH 10.14.

Inmediatamente se toma la muestra de tiempo cero, vaciando 1 ml de la mezcla sobre 0.2 ml de ácido metafosfórico al 24 %.

El resto de la mezcla se incuba a 37°C durante 30 minutos, a cuyo término se detiene la reacción agregando 0.3 ml de ácido metafosfórico al 24 %.

Después de centrifugar, se toma 0.5 ml de los sobrenadantes y se trata con 4.5 ml de reactivo sulfúrico-fosfórico y 0.25 ml de solución al 3 % de α isonitrosopropiofenona. Se calienta en baño de agua hirviente 1 hora. Se enfría en hielo, en la oscuridad, 10 minutos. Los colores se leen a 540 milimicrones.

La muestra del tiempo cero mide la concentración de urea preformada en el plasma sanguíneo, cantidad que se resta a la concentración de urea determinada después de la reacción enzimática.

b) *En el hígado.* A partir de un homogenizado 1 : 10 en solución isotónica de ClNa, se preparan diluciones 1 : 400 a 1 : 1000. La activación de la enzima se efectúa como en el plasma. La actividad arginásica se mide en un período de 10 minutos, con la técnica antes descrita.

c) *Expresión de los resultados.* La reacción sigue un curso lineal para concentraciones de urea comprendidas entre 0.2 y 2.4 μ moles por ml. Esta última cantidad equivale a la hidrólisis del 10 % de la cantidad inicial de sustrato.

Se define como *unidad de arginasa* la actividad que en las condiciones descritas, libera 1 μ mol de urea por minuto. Los resultados finales se expresan en unidades por ml de plasma o en unidades por g de hígado fresco.

Resultados

Las lesiones hepáticas producidas en la rata por la ingestión de 0.2 ml de CCl_4 tienen una intensidad variable, pero aparecen más homogéneas que las obtenidas por inyección del tóxico. El examen histopatológico del hígado revela, a las 6 horas, pequeños focos de necrosis y degeneración grasa en torno de la vena centrolobulillar. Estos focos necróticos orlados de células lipofágicas, se extienden y unen dejando pequeños islotes de parénquima normal. Este aspecto persiste hasta las 72 horas,

en que se inicia la remoción del tejido necrosado y la regeneración de las células hepáticas.

Los animales pierden peso inicialmente; en algunos de ellos se desarrolla una ictericia intensa. Al cabo de una semana, la mayoría están completamente recuperados. La mortalidad en la serie fué 4 %.

Actividad arginásica del plasma sanguíneo. La actividad arginásica del plasma de ratas no intoxicadas, mantenidas en su régimen alimenticio habitual es prácticamente nula y no es influída por la ingestión de 0.3 ml de aceite de oliva (Tabla I).

den equivalente a la de un homogeneizado de hígado normal al 10 %. Posteriormente la arginasa del plasma desciende con rapidez hasta las 72 horas y luego lentamente hasta recuperar el nivel original a las 120 ó 162 horas (Fig. 1 y Tabla II).

Las líneas verticales en los puntos de la Fig. 1 representan las amplitudes de variación individual de la arginasa plasmática en las diversas horas. Aun cuando las variaciones son considerables, dependiendo de la resistencia al tóxico, las diferencias entre los grupos son tan grandes que sus amplitudes no se sobreponen. Se consideró

TABLA I

Actividad arginásica del plasma sanguíneo de ratas no intoxicadas

Grupo	Nº de ratas	Unidades/ml de plasma	
		Media	Rango
1. Normales	26	0.011	0.00-0.08
2. Testigos que ingieren 0.3 ml aceite de oliva	9	0.003	0.00-0.02

De 35 animales examinados, la actividad arginásica del plasma estaba ausente en 25. En los restantes se encontró una actividad mínima. Reuniendo todos estos resultados, se obtiene un promedio de 0.01 U/ml, cifra influída por el hallazgo excepcional de una cantidad de 0.08 U/ml en un animal aparentemente normal.

En los animales intoxicados con CCl_4, la actividad arginásica del plasma sube y es fácilmente medible 6 horas después de la administración del tóxico, alcanzando un valor promedio 100 veces superior al normal. La arginasa del plasma aumenta rápidamente en las horas siguientes, hasta un promedio máximo de 40.4 U/ml a las 36 horas, concentración que es de un or-

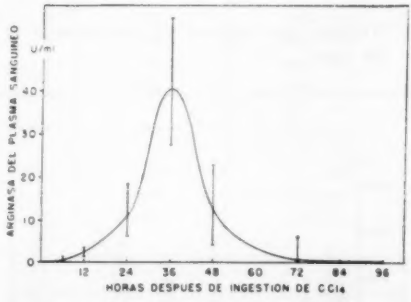


FIG. 1.—*Aparición y niveles de la arginasa del plasma sanguíneo de ratas después de la administración oral de tetracloruro de carbono. La curva corresponde a los valores promedios de cada grupo horario. Las líneas verticales indican la amplitud de las fluctuaciones individuales.*

TABLA II

Actividad arginásica del plasma sanguíneo de ratas intoxicadas por administración oral de tetracloruro de carbono

Horas después de ingestión de CCl_4	Nº de ratas	Unidades/ml de plasma	
		Media	Rango
0	35	0.01	0.00- 0.08
6	5	1.03	0.39- 1.52
12	5	3.02	1.56- 3.88
24	6	10.93	6.24-18.60
36	9	40.46	27.10-57.10
48	9	11.90	3.70-23.30
72	15	0.96	0.00- 6.45
96	9	0.07	0.02- 0.14
120	5	0.02	0.00- 0.05
162	5	0.01	0.00- 0.03

TABLA III

Actividad arginásica del hígado de ratas no intoxicadas

Grupo	Nº de ratas	Unidades/g hígado fresco	
		Media	Rango
1. Normales	15	565	468-685
2. Testigos que ingieren 0.3 ml aceite de oliva	13	477	368-542

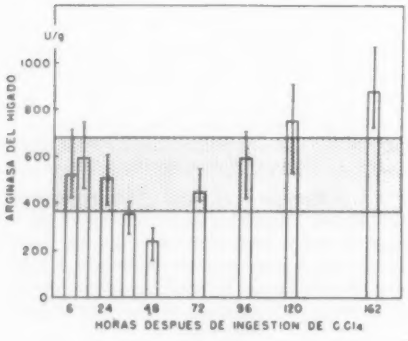


FIG. 2. — *Variación de los niveles de arginasa en el hígado de rata después de la administración oral de tetracloruro de carbono. En las columnas se indica el promedio y la amplitud de las fluctuaciones individuales. El rectángulo sombreado encierra los valores encontrados en la rata normal.*

innecesario un mayor análisis estadístico.

Actividad arginásica del hígado. En la Tabla III, se muestra la actividad arginásica del hígado de las ratas no intoxicadas o que recibieron aceite de oliva. El promedio del conjunto es 524 U/g tejido fresco. La amplitud de las variaciones normales corresponde a la zona sombreada de la Fig. 2.

En los animales intoxicados con CCl_4, la actividad del hígado permanece normal en las primeras horas, pero desciende a las 36 horas y alcanza un valor medio mínimo a las 48 horas, cuya magnitud es la mitad del promedio normal (Fig. 2 y Tabla IV). Más tarde la actividad enzimática se recupera: se normaliza a las 72 horas y continúa subiendo en los períodos siguientes hasta niveles superiores al promedio normal. La tendencia de la variación, contrapuesta a la del plasma, es claramente definida, a pesar de las amplias fluctuaciones individuales.

Localización de la arginasa en la sangre de ratas intoxicadas. Los eritrocitos centrifugados provenientes de la sangre de ratas intoxicadas con CCl_4, cuyo plasma posee una elevada actividad arginásica, presentan una actividad enzimática 10 a 20 veces inferior a la del plasma correspondiente.

Con el objeto de establecer si esta actividad arginásica eritrocítica es inherente a los eritrocitos o proviene del plasma ocluído entre las células, se lavaron los glóbulos 3 veces con volúmenes iguales de solución salina isotónica o de plasma de rata normal, exento de arginasa.

La determinación de la actividad arginásica de los eritrocitos, antes y después de estas manipulaciones, muestra que la enzima pasa rápida y totalmente a los líquidos de lavado. Los resultados de la Tabla V indican que la arginasa de los eritrocitos proviene del plasma adherido a la superficie celular.

Evolución de la urea sanguínea. La concentración de la urea en el plasma de estos animales se mide simultáneamente al determinar su actividad arginásica (tiempo cero).

Las cifras registradas en la Tabla VI, demuestran que la urea plasmática se eleva en el curso de la intoxicación hasta alcanzar valores máximos en los

TABLA IV

Actividad arginásica del hígado de ratas intoxicadas por administración oral de tetracloruro de carbono

Horas después de ingestión de CCl_4	Nº de ratas	Unidades/g hígado fresco	
		Media	Rango
0	28	524	368- 685
6	4	520	420- 714
12	5	594	466- 748
24	6	507	394- 612
36	4	357	274- 408
48	4	241	158- 286
72	4	448	410- 547
96	5	593	414- 709
120	5	733	529- 915
162	6	882	725-1070

TABLA V

Arginasa en el plasma y en los eritrocitos de una rata 36 horas después de la administración de tetracloruro de carbono. Efecto del lavado de los eritrocitos con plasma de rata normal

Material	Actividad arginásica
1. Plasma sanguíneo	27.10 U/ml
2. Glóbulos rojos	2.40 U/ml
3. Glóbulos lavados 3 veces con plasma normal	0.08 U/ml
4. Lavados provenientes de 1 ml de glóbulos	2.30 U

TABLA VI

Amplitud de la variación de la urea sanguínea en ratas normales e intoxicadas con tetracloruro de carbono

Grupo	g por litro
I. No intoxicadas	0.18-0.40
II. Intoxicadas:	
6-12 horas	0.30-0.42
24 horas	0.40-1.30
36 horas	0.60-1.70
48 horas	0.48-0.80
72 horas	0.28-0.50
90-120-162 horas	0.25-0.50

períodos en que es también máxima la actividad arginásica del plasma. La conocida acción del CCl_4 sobre el tejido renal, en el cual provoca procesos degenerativos de la porción distal del nefrón, es la causa a la cual se imputa la elevación de la uremia ([11]). Sin embargo, es evidente que la presencia de grandes cantidades de arginasa en la sangre puede ser un factor importante en la elevación de la urea.

La concentración de la urea plasmática no alcanza una magnitud suficiente para afectar la determinación de la arginasa ([14]). Hemos observado que concentraciones de urea hasta 8.0 μ moles/ml en la mezcla de incubación (equivalentes a una uremia de 2.40 g por litro en el plasma original) no ejercen influencia sobre la velocidad de la reacción enzimática.

Efecto de la dilución del plasma sobre la actividad arginásica. Midiendo la actividad arginásica del plasma en condiciones en que la hidrólisis del sustrato se aproxima al 10 % de la cantidad inicial, se ha observado que la dilución de plasmas ricos en arginasa permite revelar en ellos un nivel de actividad enzimática que excede en un 20-30 % al de los mismos plasmas no diluídos. Este hecho sugiere la existencia de un factor inhibidor cuya naturaleza y mecanismo de acción no se han investigado.

Discusión

Los experimentos aquí relatados demuestran que la intoxicación aguda por CCl_4 en la rata, causa la aparición de arginasa en el plasma sanguíneo.

En los plasmas ricos en arginasa se ha podido revelar una activación de la enzima, por dilución. Esto sugiere la presencia de un inhibidor en el plasma original.

El aumento de la arginasa plasmática es rápido y progresivo después de la administración de CCl_4, alcanzando su nivel más alto a las 36 horas, período en el cual las lesiones necróticas del hígado son muy intensas. La arginasa desciende posteriormente y desaparece del plasma al término de la regeneración. Observaciones similares han realizado Ugarte y col. ([21]) en el perro. Los efectos descritos se obtuvieron empleando 0.1 ml de CCl_4 por 100 g de rata por vía oral, dosis con la cual los efectos son relativamente constantes y más regulares que cuando el tóxico se ad-

ministra por vía subcutánea o intraperitoneal.

La forma de la curva que describe la variación de la arginasa plasmática después de la ingestión de CCl_4 (Fig. 1) es típica, y no es afectada por las amplias fluctuaciones que traducen la diversa resistencia individual a la acción del tóxico. La ausencia de arginasa en el plasma de las ratas no intoxicadas da especial claridad y significado a esta respuesta enzimática, que parte prácticamente desde un nivel cero.

La actividad arginásica del hígado varía diseñando una curva inversa a la de la arginasa plasmática. El mínimo de actividad arginásica del hígado a las 36 y 48 horas es contemporáneo con la presencia de niveles máximos en el plasma sanguíneo. La forma de la curva construída con los valores promedios y fluctuaciones de la arginasa hepática (Fig. 2) es claramente definida, aun cuando las amplitudes de variación en períodos sucesivos se sobreponen parcialmente.

En las etapas finales de la observación, cuando el hígado está recién regenerado, la actividad arginásica de este órgano llega a superar los márgenes de variación comunes sugiriendo la posibilidad de que esta enzima tenga una función reguladora, limitando la expansión de la masa de tejido durante el crecimiento normal.

La determinación de la arginasa en el plasma sanguíneo podría tal vez servir en clínica humana para detectar alteraciones destructivas del parénquima hepático. Sin embargo, la existencia de arginasa en el citoplasma de los eritrocitos humanos [4], requiere la adopción de precauciones cuidadosas, para excluir una transferencia de la arginasa eritrocítica hacia el plasma, por fenómenos de hemólisis intrínsecos o extrínsecos.

En cuanto al mecanismo por el cual penetran cantidades considerables de arginasa del hígado al plasma, aparece vinculado a la acción tóxica del CCl_4 sobre la célula hepática. El estudio de las alteraciones del hígado causadas por el CCl_4 ha revelado los siguientes efectos: 1º) Modificación de la concentración de varios componentes celulares: aumento del contenido de grasa, disminución del glicógeno y, en menor grado, del N total. La célula hepática pierde coenzima A, piridín-nucleótidos, citocromo C, pirofosfato de tiamina y ATP, de preferencia en la fracción mitocondrial. 2º) Alteración de diversas actividades enzimáticas coordinadas: descenso de la respiración, desacoplamiento de la fosforilación oxidativa, menor capacidad oxidativa para numerosos sustratos que requieren DPN, la que se restaura por adición de la coenzima. 3º) Pérdida de la función mitocondrial; tumefacción de las partículas que se hacen esféricas y traslúcidas, llegando a desaparecer, aumento de la susceptibilidad a las alteraciones bioquímicas causadas por el envejecimiento y aumento de la permeabilidad que determina la migración hacia el citoplasma de los piridín nucleótidos, pirofosfato de tiamina y ATP, con aumento relativo del ADP [5, 6, 8–10, 12, 16–18].

Recknagel y Anthony [15] han señalado que 20 horas después de la ingestión de CCl_4 se producen 2 lesiones mitocondriales típicas: a) pérdida de la acción estimulante del 2-4 dinitrofenol sobre la oxidación del α-cetoglutarato y del glutamato, y b) transformación del carácter de la actividad ATPásica, disminuyendo la estimulada por dinitrofenol y aumentando considerablemente, en cambio, la que depende de Mg^{++}. Thiers y Reynolds [20] encuentran un aumento del Ca intramitocondrial que llega hasta 10 veces el valor normal a las 16 horas después de la intoxicación.

Basándose en estas observaciones, muchos autores [12] consideran que el ataque primario del CCl_4 se efectúa sobre

la estructura y función de las mitocondrias, condición que determina la degeneración y necrosis de la célula hepática. La grasa se acumularía como consecuencia de la inhibición de la oxidación de los ácidos grasos demostrada en el caso particular del octanoato y atribuída a la falta de coenzima A y a la depresión de la reacción activadora inicial de los ácidos grasos ([17]). Sin embargo, otros autores consideran que la lesión mitocondrial es tardía y por lo tanto, secundaria a otra acción bioquímica no definida ([15]).

Observaciones anteriores de Cabello, Prieto y Prajoux ([3]) relativas a la distribución intracelular de la arginasa en el hígado de la rata, demuestran que un 80 % de la actividad arginásica recuperable está ligada a las partículas y que, el 50 % del total se encuentra en las mitocondrias.

Estos hechos sugieren la posibilidad de que los altos niveles de arginasa plasmática producidos en la rata por la administración de CCl_4 sean efecto de una alteración de la permeabilidad y estructura mitocondrial, lo que causa la difusión de la arginasa al citoplasma del hepatocito, al través de cuya membrana dañada la enzima encuentra acceso a los sinusoides lobulillares.

Conclusiones

1. La sangre de la rata normal carece de arginasa o posee sólo indicios de esta enzima.

La intoxicación de la rata con CCl_4 determina la entrada de gran cantidad de arginasa del hígado a la sangre. La actividad enzimática aparece localizada en el plasma sanguíneo.

2. La más alta concentración de arginasa plasmática se encuentra 36 horas después de la administración del tóxico y coincide con la mayor intensidad de la necrosis hepática. Después la actividad enzimática disminuye, desapareciendo a las 120 horas, en la mayoría de los casos.

3. El contenido de arginasa del hígado desciende hasta un 50 % del valor inicial a las 48 horas, y luego sube hasta exceder el promedio normal al término de la fase regenerativa.

4. La concentración de la urea sanguínea asciende en los animales intoxicados hasta alcanzar un máximo a las 24-36 horas después de la intoxicación.

5. Considerando las acciones del CCl_4 sobre la estructura y función del hepatocito y la prevalencia de la actividad arginásica en la fracción mitocondrial, la aparición de una elevada concentración de arginasa en el plasma durante la necrosis hepática puede hallarse relacionada, en cierto grado, con las alteraciones de la permeabilidad mitocondrial y la desorganización de la membrana celular.

Agradecimientos

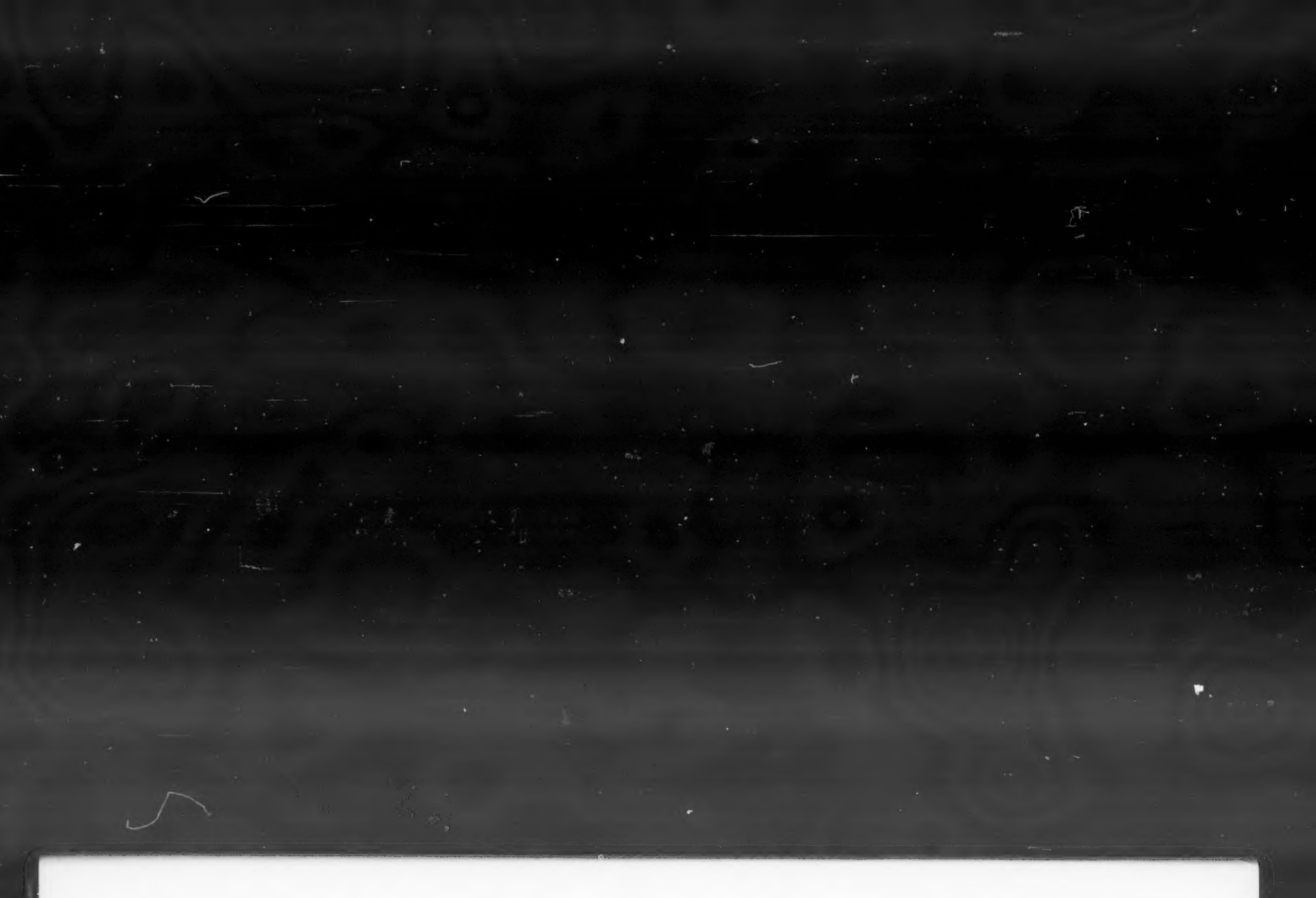
Los exámenes histológicos del hígado fueron efectuados por el Dr. Carlos Aliaga, en el Instituto de Anatomía Patológica del Hospital San Borja (Profesor H. Rodríguez), cuya colaboración agradecemos.

Expresamos nuestro reconocimiento a la Srta. María Plaza por su eficaz ayuda técnica.

Summary

The oral administration in a single dose of 0.2 ml of carbon tetrachloride to 200 ± 20 g rats causes hepatic necrosis and subsequent liberation of liver arginase into the blood stream.

Normal rats have none or negligible arginase activity in the blood plasma (mean: 0.01 units per ml, Table 1) and no detectable arginase in the red cells. However, arginase activity raises to very high levels in the blood plasma of rats fed with carbon tetrachloride, the maximum value (mean: 40.5 units per ml)



FE DE ERRATA

Nº 3, vol. X, 1960, page 155

FIG. 1: it reads: "*Black symbols*: values obtained when the conditioned stimulus was applied without insulin injection".

It should read: "*Black symbols*: values obtained when insulin was injected – *White symbols*: values obtained when the conditioned stimulus was applied without insulin injection".

Nº 3, vol. X, 1960, page 167 6th line

Left, it reads: "ministration (Table II, Fig. 1). At this".

It should read: "plasma (Table V). After reaching its".

being reached 36 hours after drug administration (Table II, Fig. 1). At this time the most severe hepatic necrosis occurs as substantiated by histological findings. The enzyme is only found in ministration (Table II, Fig. 1). At this maximal level, the arginase activity of plasma rapidly decreases becoming null in most cases after 120 hours of intoxication, when the liver is regenerated. Therefore it may be concluded that the presence of detectable amounts of arginase in the blood plasma constitutes an index of liver necrosis, the level attained being correlated with the degree of hepatic injury.

As a clear counterpart of the above described changes in blood arginase, the content of this enzyme in liver, progressively decreases and falls to half of the initial level after 48 hours of intoxication (Tables III, IV, Fig. 2). Afterwards, the liver arginase level goes up to normal and even higher values at the end of the regenerative phase. This observation is consistent with the assumption of arginase being implicated as a regulatory factor in the normal limitation of tissue growth.

Blood urea increases in intoxicated animals and a maximum value is reached 24-36 hours after carbon tetrachloride ingestion (Table VI). This effect, which parallels the rise of arginase in plasma, has commonly been ascribed to kidney injury. The possibility that it may be also dependent on the high arginase activity of blood must be contemplated.

The available evidence on the effects of carbon tetrachloride on structure and function of the hepatocyte indicates an early and probably primary mitochondrial injury. The permeability changes and swelling of these particles can be so profound as to determine their disappearance as morphological entities. This results in the release to the cytoplasm of several cofactors normally bound to particle structure and the modification of the enzymatic pattern and activities of mitochondria. As intracellular arginase has been found mainly localized in the mitochondrial fraction, the large and rapid increase of arginase level in blood plasma after carbon tetrachloride administration may depend on toxic damage to mitochondria and to the cell membrane, followed by diffusion of the contents of necrosed liver cells to lobular sinusoids and capillaries.

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EFFECT OF ASCORBIC ACID ON EXPERIMENTAL ATHEROSCLEROSIS IN THE CHICKEN (*)

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Ascorbic acid has been reported to decrease aortic atherosclerosis in cholesterol-fed rabbits [1] and its use is being advocated for the treatment of human atherosclerosis [2]. Nevertheless, since rabbits are herbivorous animals, it was felt that a study of the effects of ascorbic acid on coronary and aortic atherosclerosis in an omnivorous species was desirable. Consequently, vitamin C was injected into cholesterol-fed chickens and its vessels examined.

Material and methods

Six week old, white Leghorn cockerels were fed commercial chick starter mash (***) during one week and placed afterwards on mash supplemented with 1 % cholesterol (****). They were then divided into three groups of 15 birds each: a) saline-injected controls; animals injected with b) 0.1 g of ascorbic acid (*****); and c) with 1.0 g of ascorbic acid. Intramuscular injections were performed five times a week. The ascorbic acid content of the injections was frequently checked with Roe and Kuether's method [3]; concentration differed with the theoretical dose by less than 1 %. The birds were sacrificed after 8 weeks on this experimental regimen; further methods of studying the animals after sacrifice as well as statistical handling of results have been described previously [4].

Results

Results are shown in tables I through III. It can be seen that chickens gained weight normally, although the injection

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(***) Manufacturer's composition: Proteins 19 %; Fats 5 %; Carbohydrates 50 %; Salts 10 %; Roughage 6 %; Vit. A 7000 U/100 g; Vit. D 1300 U/100 g; Aureomycin 250 mg/100 g.

(****) Cholesterol USP, Nutritional Biochemicals, Cleveland, Ohio.

(*****) Ascorbic acid was kindly provided by Roche, S. A. Argentina as "Redoxón".

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TABLE I

Findings in cholesterol-fed cockerels

Group	Nº of birds	Comb-index (g/1000 g)	Testicles (g/1000 g)	Adrenals (mg/1000 g)	Pituitary (mg/1000 g)	Initial body weight (g)	Final body weight (g)	% of increase in body weight
1) Saline injected ...	11	16.540 ± 1.000	5.229 ± 0.953	199.9 ± 68.9	3.476 ± 0.504	422 ± 19.999	1387 ± 18.000	228.67
2) Ascorbic acid 0.1 g	12	19.131 ± 2.081	4.725 ± 1.002	137.8 ± 28.4	5.169 ± 0.517	397 ± 23.780	1212 ± 50.000	205.28
3) Ascorbic acid 1.0 g	12	16.964 ± 3.904	3.571 ± 0.901	152.3 ± 11.5	5.010 ± 0.680	391 ± 21.390	1086 ± 37.000	177.74
	"t"	1 vs 2 1.110	0.365	0.838	2.348 (*)			
		1 vs 3 0.540	1.260	0.687	1.440			

(*) $p < 0.05$.

TABLE II

Atherosclerotic lesions found in chickens

Group	Nº of birds	Aortic macroscopic lesions						Coronary microscopic findings grade %					
		O to +	++	+++	++++	x^2 (*)	p	0-5	5.1-10	10.1-20	> 20	x^2	p
1) Saline injected ...	10	8	2	1	0			2	0	2	6		
2) Ascorbic acid 0.1 g	12	6	4	1	1	1.244	> 0.20	2	1	1	8	0.040	> 0.95
2) Ascorbic acid 1.0 g	11	5	4	2	1	1.287	> 0.20	2	2	1	6	0.016	> 0.98

(*) Birds are classified as negative (those having none to minimal lesions) or positive. Comparisons are made vs corresponding saline injected controls

TABLE III

Chemical determinations in chickens

Group	Nº of birds	Cholesterol		
		Blood mg/100 ml	Nº of birds	Aorta mg/100 g
1) Saline injected	11	305.00 ± 25.9	10	242.80 ± 41.24
2) Ascorbic acid 0.1 g	11	260 ± 27.1	9	273.55 ± 28.75
3) Ascorbic acid 1.0 g	12	272 ± 26.9	11	302.8 ± 25.04
		"t" 1 vs 2 1.356		0.615
		1 vs 3 0.915		1.251

of 1 g of ascorbic acid somewhat depressed body weight. Vitamin C was not able to modify aortic nor coronary atherosclerosis and had no effect on blood or aortic cholesterol. No consistent endocrine changes could be adscribed to the injections, although birds receiving 0.1 g of vitamin C had heavier pituitaries than their controls.

Discussion

Our results clearly show that ascorbic acid given parenterally, does not modify aortic or coronary atherosclerosis in cholesterol-fed cockerels. Opposite results have been reported in cholesterol-fed rabbits, namely, prevention of aortic atherosclerosis (1); whether this is due to species differences or to other factors can not be presently stated, although it must be stressed that Flexner et al. (5) have already reported negative results in rabbits injected with vitamin C.

Summary

Cholesterol-fed chickens were given 0.1 or 1.0 grams of ascorbic acid by intramuscular injections five times a week. Vitamin C was without effect in modifying the incidence of aortic or coronary atherosclerosis.

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ANTAGONISM BETWEEN THYROID AND GONADS UPON THE HAIR CYCLE IN C_3H/Ba MICE (*)

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The growth of hair in rats and mice is cyclic, every hair follicle has alternate periods of growth and rest [2, 7]. Hair growth is not uniform, but there are centers of growth from which it spreads in waves all over the body [5, 7].

Several endocrine influences modify these hair cycles. The most interesting alteration is the one produced by gonadectomy in mice [9, 12] and either by adrenalectomy [1, 9, 14, 17] or hypophysectomy [10, 16], in rats and mice. In them, all the resting hair follicles are stimulated after operation and all the denuded area is rapidly and simultaneously covered by a coat of hair [9, 12]. This action is inhibited by estrogens [8, 9, 13], androgens [11, 13] and corticoids [9, 13, 17].

Thyroidectomy retards hair growth in rats [4, 5] and mice [11] and the animals are covered by hair at a slower rate. The thyroid hormones accelerate hair growth in hypothyroid [4] and normal [3] rats.

This work was devised with the object to find out if thyroidectomy would be effective in retarding the rapid and simultaneous growth of hair induced after gonadectomy.

Material and methods

The experiments were performed in male C_3H/Ba mice, weighing from 20 to 30 g, belonging to the subline of the inbred strain of mice, bred under the direction of Dr. Carlos E. Epper, at the Institute of Oncology, University of Buenos Aires. The animals were selected in such a way that littermates were equally distributed in the different groups.

Thyroidectomies were made by intraperitoneal injection of 100 microcuries

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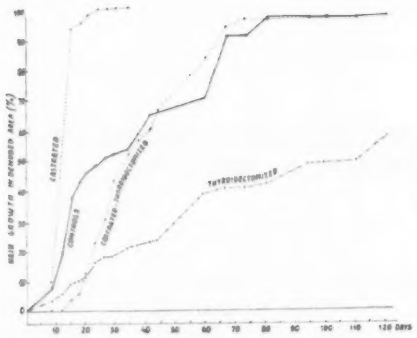

FIG. 1.

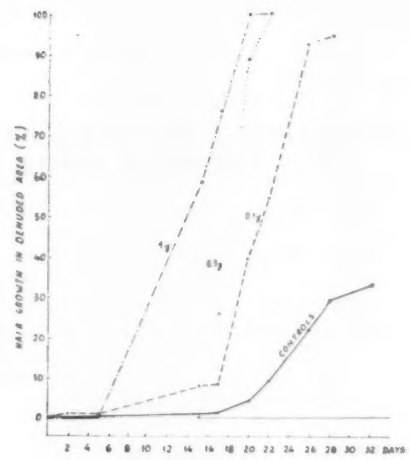

FIG. 2.

of I^{131}. After an interval of 20 days, in which period the destruction of the thyroid was completed, the experiment began and every mice was clipped and some groups of animals were castrated surgically.

Every mice was clipped with an electric clipper number 0000, over an area extending along the back, from the neck to the base of the tail, at the beginning of every experiment.

The clipped area was observed 2 or 3 times a week along the whole of each experiment and the percentage of the denuded area covered by hair was estimated by inspection every time. With the average data obtained in the measurements in every group, Figures 1 and 2 were drawn.

Several groups of castrated-thyroidectomized mice were injected intraperitoneally daily for 30 days, with an aqueous solution of sodium l-thyroxin, in concentrations of 0.1, 0.3 or 1 micrograms in the 0.2 cc injected in every animal.

At the end of the experiment the mice were killed with ether. A complete autopsy was performed and the prostate, seminal vesicles, testicles, pituitary, adrenals, thyroid, thymus and salivary glands were weighed. A histological study was performed of the skin, testicles, thyroid and pituitary.

The mice were weighed before the operations (initial weight) and before they were killed (final weight).

In every experiment a statistical study of the results obtained was made, and the average, quadratic deviation and standard error were calculated.

RESULTS

In the first experiment the effects of castration and thyroidectomy upon the growth of hair were compared in 4 groups of C_3H/Ba mice: 1) castrated mice, 2) thyroidectomized mice, 3) castrated-thyroidectomized mice and 4) normal mice.

Thyroidectomy was produced by 100 microcuries of I^{131} given 20 days before the beginning of the experiment, so the mice would be in thyroid insufficiency at that time. Castration and clipping were carried out the same day the experiment began. The data on the growth of hair in these different groups is shown in Graph 1.

The hair follicles of the back of these animals were in the resting period and

no hair waves were seen macroscopically.

In control animals a normal cyclic rhythm was observed and the denuded area was covered gradually by the hair wave .After 70 days nearly all the backs were covered by hair.

The backs of the thyroidectomized mice were also covered by a hair wave but it was so slow that at the end of the experiment, 120 days after clipping, they were still not completely covered by hair in any of the animals.

In castrated mice the hair grew rapidly and simultaneously in all the backs and they were completely covered by hair 20 days after clipping.

In castrated-thyroidectomized mice the hair grew in waves similar to those of normal controls and the clipped backs were covered by hair in the same interval of time, as seen in figure 1. At 70 days after clipping practically all the backs were covered by hair.

The differences in the growth of hair in these 4 groups of animals are clearly shown in figure 1. There it can be seen the slow hair waves of thyroidectomized mice, the rapid and homogeneous hair growth in castrated mice, and the similarity of the hair waves in castrated-thyroidectomized and in normal mice.

In table I the weight of the animals and their organs are presented. There was a decrease of body weight in thyroidectomized mice either castrated or not. In castrated mice there was atrophy of prostate and seminal vesicles, decrease in the weight of the thyroid and increase in the weight of the thymus. In thyroidectomized mice there was decrease in the weight of prostate and seminal vesicles, increase in the weight of pituitary and adrenals and decrease in the weight of thymus.

It was evident that thyroidectomy by I^{131} diminished the rate of hair growth in normal and castrated mice. To ascertain that these results were due to thyroid insufficiency and not to the radiation delivered by the radioisotope, or to nutritional factors as suggested by weight loss, we devised Experiment 2. Here substitution therapy with l-thyroxine was given to castrated-thyroidectomized mice to find out if the rapid and simultaneous hair growth of recently castrated mice could be restored.

Thus, in 4 groups of male C_3H/Ba mice, radio-thyroidectomy was made by 100 microcuries of I^{131} and 20 days later the animals were castrated and their backs were clipped. Sodium l-thyroxine was injected intraperitoneally daily for 30 days to 3 groups of mice in the following way: group 1 - 1 microgram daily, group 2 - 0.3 micrograms daily, group 3 - 0.1 micrograms daily and group 4 - controls. The results are presented in figure 2.

We can see that in every group where l-thyroxine was administered hair growth was greatly accelerated as compared with the castrated-thyroidectomized controls, and that was due to the restoration of the rapid and simultaneous hair growth of castrated mice. The difference in the hair growth in these different groups is mainly due to the latency period before the appearance of the diffuse hair growth, being shorter when the dose administered was higher. With 0.3 or 1 micrograms daily the hair growth was diffuse in all the mice, with 0.1 micrograms daily a very rapid hair wave was found in some. We think that the physiological dose of l-thyroxine which can restore the hair growth in castrated animals by intraperitoneal route is 0.3 micrograms daily.

In Table II the results on body and organ weights are presented. Body weight in castrated-thyroidectomized mice was maintained with 0.1 micrograms daily and increased with 0.3 and 1 micrograms daily. The administration of l-thyroxine did not change the

TABLE I

Influence of thyroidectomy on normal and castrated C_3H mice

Groups	Animals		WEIGHT OF ORGANS (mg ± S.E.)								
	Nº	Weight g ± S.E.	Anterior Prostate	Posterior Prostate	Coagulant glands	Seminal vesicles	Testicles	Thyroid	Adrenals	Pituitary	Thymus
Normal	5	31.8 ± 2.8	17.2 ± 2.1	20.4 ± 2.3	20.5 ± 2.3	152 ± 6.1	143 ± 6	4.5 ± 1.0	6.7 ± 1.3	3 ± 0.8	23 ± 2.4
Castrated	6	33.8 ± 2.6	2.2 ± 0.6	2.0 ± 0.6	2.2 ± 0.7	5.9 ± 1.1	—	3.7 ± 0.8	7.2 ± 1.2	2.8 ± 0.7	32 ± 2.6
Thyroidectomized	13	29.8 ± 1.6	16.2 ± 1.2	13.5 ± 1.1	18.1 ± 1.3	101 ± 3.0	143 ± 3.6	—	8 ± 0.8	4.3 ± 0.6	13 ± 1.1
Castrated-Thyroidectomized	14	24 ± 1.3	2.3 ± 0.4	3.1 ± 0.5	1.5 ± 0.3	5.7 ± 0.6	—	—	8.7 ± 0.8	3.7 ± 0.5	16 ± 1.1

TABLE II

Action of l-thyroxine in castrated - thyroidectomiced C_3H mice

L-Thyroxine Micrograms Daily	ANIMALS			WEIGHT OF ORGANS (mg ± S.E.)								
	Nº	Weight g ± S.E.		Anterior Prostate	Posterior Prostate	Coagulant glands	Seminal vesicles	Adrenals	Pituitary	Thymus	Submaxilary and Sublingual	Parotid
		Initial	Final									
0	10	23.2 ± 1.6	22.2 ± 1.2	7.3 ± 0.9	6.4 ± 0.8	4 ± 0.7	11.3 ± 1.1	10.3 ± 1.1	3.7 ± 0.6	32 ± 1.9	71 ± 2.8	76 ± 2.9
0.1	9	23.3 ± 1.7	23.6 ± 1.7	8.5 ± 1.0	6.9 ± 0.9	3.4 ± 0.6	12.5 ± 1.2	9.5 ± 1.1	2.5 ± 0.6	48 ± 2.5	65 ± 2.9	95 ± 3.4
0.3	8	21.8 ± 1.7	24.2 ± 1.8	6.7 ± 0.9	6 ± 0.9	4 ± 0.7	8.9 ± 1.1	8 ± 1.1	2.5 ± 0.6	40 ± 2.4	67 ± 3.1	103 ± 3.8
1.0	8	21.6 ± 1.8	24.3 ± 1.9	5.8 ± 0.9	7.1 ± 1.0	5.3 ± 0.9	11.3 ± 1.3	9.6 ± 1.1	2.6 ± 0.6	49 ± 2.6	77 ± 3.3	107 ± 3.8

weight of testicles, prostate and seminal vesicles, decreased the weight of pituitary and adrenals and increased the weight of the thymus and parotid glands.

If we compare the results of Table I and II, we observe that thyroid hormones have a stimulating action upon prostate and seminal vesicles only if testicles are present. It is also clear that thyroidectomy increases the weight of pituitary and adrenals and this effect is inhibited by l-thyroxine. Moreover, castration decreased the size of thyroids. Besides, it is apparent that thyroid hormones exert a trophic action upon thymus and parotids.

From the results presented it is concluded that thyroidectomy retards hair growth in normal and castrated mice and the administration of l-thyroxine restores it to normal. Besides, the experiments, show that testicles and thyroid had an opposite effect on the control of hair cycles, so when one factor is suppressed the balance is disrupted towards the opposite side. Thus, after castration a rapid and simultaneous hair growth ensues and after thyroidectomy hair growth waves become very slow. When both opposite factors are suppressed the balance is restored again and the resulting hair growth is very similar to that of normal mice.

Discussion

Every hair follicle has alternate periods of growth and rest, in every growth period a new hair grows ([2, 7]). This period of growth is divided according to the histological appearance into several phases: anagens 1, 2, 3, 4, 5 and 6, and its duration is 21 days in C_3H/Ba mice. The resting period is called telogen, it is very variable in length in normal mice and it increases in every hair cycle, that is to say as the animal grows old ([18]).

Every hair cycle begins in a limited part of the skin as a transversal band running symmetrically across the body, where hair follicles are in the same period of stimulation. These bands move slowly backwards, sometimes forward, and cover the rest of the skin gradually, the region where they have already passed being in resting period again. As C_3H/Ba are pigmented mice, these bands are noticed by the dark pigmentation of the skin preceding hair growth and its movement is easily observed. Thus, every complete hair coat is produced by a single hair cycle that covers all the skin surface of the animal, slowly and gradually, in an orderly and symmetrical way.

Gonadectomy disturbs this hair cycle completely, immediately after the operation. The alteration consists in the immediate stimulation of resting hair follicles, so that the hair cycle begins and ends at the same time in all those follicles and the animal is thus covered in 21 days by a new coat of hair in a diffuse way. If all the clipped hair follicles are in telogen phase when the animal is castrated, the growth of hair is simultaneous in all the denuded surface, at 15 days all the skin becomes dark and at 21 days the hairs of the new coat are as long as the non-clipped ones. We called this pattern of hair growth "diffuse growth of hair". Gonadectomy does not alter the individual growth of each hair ([18]).

When in the denuded area there is a dark band of active hair follicles belonging to a hair wave, they continue their normal growth and it is finished in that place before the appearance of the diffuse wave. Thus one part of the clipped area is covered by the wave already active, but the remaining surface that was in telogen at castration is covered by the diffuse growth. In summary, immediately after castration hair follicles in telogen phase are excited and produce a diffuse and simultaneous growth, bands of hair follicles

in late anagen continue their normal growth which ends before the diffuse growth. In any case, 21 days after castration the animal is covered by a new coat of hair.

When the diffuse wave is finished the cyclic rhythm in slow waves appears again. This could be explained by an adaptation of hair follicles or by the inhibitory action of other endocrine glands such as pituitary or adrenals.

Thyroid insufficiency produced in our experiments a marked retardation in the growth of hair, either in the gradual waves of normal mice or in the diffuse growth of castrated mice. Besides, substitution therapy with l-thyroxine restored the diffuse growth in castrated-thyroidectomized mice. This confirms the stimulating physiological action exerted by thyroid hormones upon the hair follicle.

These experiments confirm our hypothesis that the hair cycle is under hormonal control [9, 10, 15, 16], even if other factors may be important. Some hormonal factors are inhibitory, as the gonadal and adrenal hormones, that inhibit the diffuse hair growth and permit the gradual stimulation of hair follicles [9, 10, 13, 15, 16]. Other hormonal factors are stimulating, as thyroid hormones and if they are absent, hair waves are very slow and the diffuse hair growth is not possible.

When some of the inhibitory factors are suppressed, either the gonads or the adrenals, the normal balance is altered, the excitatory factors predominate and they produce the diffuse hair growth. If we suppress the thyroid also, the balance is obtained again and there are normal waves. The reappearance of normal waves in the second hair cycle after castration or adrenalectomy could be explained as an adaptation through the pituitary and the other inhibitory glands, either adrenal or gonad.

When the thyroid, a very important stimulating factor, is suppressed, hair growth is very slow, that is to say the balance is broken because the inhibitory factors, adrenals or gonads, predominate.

The fact that hypophysectomy produces diffuse hair growth [16] immediately after operation, would prove that the pituitary is principally inhibitory and when it is removed the balance is disrupted in favor of stimulating factors. We do not know which is the stimulating factor that acts in absence of the anterior pituitary. The work of Eser [6] points to the hormones of the posterior pituitary, but we have not been able so far to confirm their findings.

The experiments reported in this paper clearly show that thyroid and testicles have an antagonistic action upon the hair cycles. The suppression of one of them produces an inbalance because the other gland predominates. posterior pituitary, but we have not been able so far to confirm their findings.

Summary

The effect of castration and thyroidectomy were compared in 40 male C_3H/Ba mice. In the normal controls hair grew following the typical hair waves. In the castrated ones the growth was greatly accelerated and the hair grew simultaneously all over the denuded back of the animals. In the radiothyroidectomized mice hair growth was very slow, but waves of growth were noticed. In radiothyroidectomized-castrated mice hair growth was identical to that of the normal mice used as controls, taking nearly the same time in covering the denuded areas.

The administration of 0.1, 0.3 or 1 micrograms of l-thyroxine daily to castrated-thyroidectomized mice restores the diffuse hair growth of castrated mice.

In short, hair growth in normal and

castrated C_3H/Ba mice is retarded by thyroid insufficiency and the thyroid and testicles have opposite actions of similar intensity upon the hair cycles of these animals.

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A NEW DIURETIC FACTOR OF HEPATIC ORIGIN

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MANY factors are involved in water diuresis and in sodium excretion (natriuresis). Neither can be attributed *simply* to variations in osmotic pressure following dilution of the blood nor to variations in glomerular filtration pressure resulting from a temporary increase in blood volume. Even the activity of the hypothalamic neurohypophysial system (considered the leading factor in diuresis because it controls the level of the anti-duiretic hormone (ADH) in the bloodstream) is not sufficient to account for all the phenomena encountered. If it were, diuresis would occur almost *immediately* following the intravenous administration of water. Water given by this route at once affects the hypothalamic neurohypophysial system, causing rapid decrease of the ADH concentration in the blood; at the same time immediate variations in osmotic pressure and changes in the volume of the blood would occur — all factors tending toward an early diuretic response. This is not the case, however. Numerous investigators [1-8] have pointed out that diuresis does not occur *immediately* after water (or, rather, its physiologic equivalent, 3 or 5 per cent glucose) has been administered intravenously even when the quantity given is as much as one third of the total blood volume (Thompson) [9]. The "delay time", or period between the time when maximal plasma dilution occurs and the time that diuresis reaches its peak, varies according to the route of administration. According to Westfall and coworkers [10], Haldane [5], Priestly [6, 7], and others, diuresis takes place 30 to 60 minutes following intravenous administration of water; 2 to 3 hours after it is given subcutaneously, and only 20 to 30 minutes following oral ingestion. (*) As Smirk [12] points out, the onset of diuresis does not occur at the moment when maximal dilution of the blood takes place. On the other

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(*) Hollander, W. Jr. and Williams, T. F. [11] reported that they had reached the conclusion that there was no significant difference between the water diuresis produced by oral and by intravenous water loading in human subjects. However, the present author feels that the probable reason that no differences were observed during their study is that long-term experiments were conducted. In such cases, the normal compensatory reactions serving homeostasis would have had the opportunity to intervene and provoke adequate diuresis, thereby producing a balance in the normal water load.

hand, the results of Ladd's studies [13] demonstrated that intravenous administration of three liters of saline solution in man did not significantly increase the urinary excretion rate, while a subject who was prehydrated by having him drink a liberal amount of water (two liters) 8 to 13 hours prior to the intravenous infusion of saline showed a marked increase in diuresis following a subsequent infusion. In addition, Blomhert et al. [14] reported that isotonic saline solution when given at night provoked diuresis if given *orally* but had no effect on urinary excretion if *infused*. Neither these findings nor the fact that the "delay time" is of shorter duration when water is taken orally than when intravenously administered are explained by any of the physiologic systems presently known.

In seeking an explanation for this apparent paradox the author formulated the problem in the following way: How does orally ingested water reach the circulatory system, and in what way does this route differ from the route taken by water parenterally administered? Water, taken orally, must first pass through the liver by way of the portal vein, while, when administered parenterally, it enters the systemic circulatory system directly. This suggested that oral ingestion of water is followed by a diuretic effect as a result of its passage through the liver. Pick [15] has already suggested that the part played by the liver in water balance is important, first of all because it can store water and secondly because diuretic and antidiuretic hormones may be produced in that organ. However, before entering the liver, orally ingested water must first pass through the gastrointestinal tract, any portion of which could also enhance its diuretic properties. Grindsberg [16], Cow [17, 18] Schmid [19] and Ambard [20] reported that they had obtained an extract from the intestine, *by maceration*, that had diuretic properties.

However, it seemed unlikely to the author that such a diuretic factor could be liberated physiologically. It seemed more probable that the intestine is simply a channel through which water passes, in addition, of course, to being the surface where absorption and early osmotic adjustements take place. The diuretic property of ingested water was through the liver causes the release or expressed differently, water in passing through the liver causes the release or production of a factor that promotes diuresis. Experiments were then undertaken to test this hypothesis.

EXPERIMENTAL PROCEDURE: The initial experiment consisted of taking 10 ml of blood from the portal vein of a dog and, at the same time, 10 ml from the hepatic vein. The entire amount of each sample (heparinized) was administered intravenously to a dog. The animal had been prepared by inserting catheters into each ureter and allowed a steady urinary flow to be established. The portal blood was injected first; it resulted in a slight increase in urinary output. When urinary flow became uniform again at previous levels, the blood from the hepatic vein was injected. Diuresis occurred again, but this time to a considerably higher degree than with the blood from the portal vein. Following the completion of this simple test, a more refined procedure was devised involving perfusion of the liver.

A cannula was inserted into the trunk of the portal vein of an anesthetized dog; the vein was then ligated below the cannula, eliminating the intestine from the intended perfusion area. Another cannula (the drain) was inserted into the main branch of the hepatic vein at a point immediately before the opening of this vein into the inferior vena cava. The liver was then perfused via the cannulas with 7 or 8

liters of aerated Tyrode's solution maintained at 38° or 39°C. In the early experiments the recovered liquid was freed of products of a vasodilatory nature prior to preparing it for testing (1) through precipitation with trichloroacetic acid. Subsequent observations revealed that when this step was not taken there were no modifications either in renal plasma flow or in glomerular filtration rate. The procedure was therefore discontinued and only the following steps were taken: 1) the recovered perfusate was centrifuged to remove the blood cells; 2) the supernatant was lyophilized to remove the water present from Tyrode's solution and 3) the dried material was dialyzed to remove the Tyrode's and all remaining salts. The remaining solution contained a residue which apparently had been produced in the liver. This preparation was used in the early, volumetric experiments performed at the Institute of Experimental Medicine, School of Medicine, Montevideo, Uruguay. More complete experiments subsequently performed at Yeshiva University, Albert Einstein College of Medicine, New York, with the invaluable advice of Prof. A. Gilman, Department of Pharmacology, will also be described.

EARLY EXPERIMENTS WITH HEPATIC SUBSTANCE: *Volumetric Experiments in Anesthetized Dogs.* Dogs were anesthetized with chloralose (*). A catheter was inserted into each ureter and the number of drops excreted were recorded. This method obviated the problem of incomplete measurement because of dead spaces, such as occurs with catheterization of the bladder, and it was possible to determine more precisely when diuresis began, when it reached its peak, and when it subsided. After signs of postoperative shock had disappeared and a steady rhythm of urinary flow had been established, a 2 ml injection (*) of the purified liver substance described above was given intravenously. Within 20 to 30 minutes diuresis began, reaching its peak in 50 to 60 minutes. Increase in the volume of urine excreted ranged from 80 per cent to 300 per cent. The dogs in this series did not receive any water by the oral route prior to the experiment, and no priming or constant infusions were given. Of the 26 experiments of this type done, results were positive in 24, negative in 1, and of doubtful significance in the other.

Experiments in Unanesthetized dogs. Normal unanesthetized dogs, without operative trauma, were placed in metabolism cages for the purpose of collecting and measuring the daily volume of spontaneous urinary excretion. They were fed measured amounts of liquid and solid food for several days until they had become accustomed to their surroundings and a regular rhythm of urinary output had been established. At this time an intramuscular injection of the liver substance was given. Some of the dogs received only one 10 ml dose; others received daily 10 ml doses of the liver substance over a period of from two to six days. (When injections of the liver substance were given on two consecutive days, the second dose often proved ineffective, probably because of the excessive diuresis that had occurred the previous day.) There were no instances of anaphylactic shock or other untoward reactions. Thirty four experiments of this type were performed; results were positive in 24, while

(*) It has been established that chloralose is the anesthetic which least interferes with urinary excretion. (De Bodo, R. C. and Bloch, H. I.) (21)

(*) The material used for testing at this stage was not re-lyophilized and the dose in ml refers to the solution recovered from the dialysis bag. Consequently, the amount of the substance administered was not known at this time.

the remaining 9 were negative. Increases in urinary excretion ranged from 100 to 300 per cent. Results of urine analysis made in connection with these experiments are not reported because the urine collected was contaminated by the stools and food. Bladder catheterization was not done in this series of experiments and so it was impossible to know whether the bladder was completely evacuated, but since these were volumetric experiments only this means that the positive results were understated, if anything.

Control Experiments. In order to determine whether this new diuretic factor is secreted also by other organs or is specifically a product of the liver, perfusions of the spleen and of loops of intestine were performed. The substance thus obtained had no diuretic effect when tested as above.

Preliminary Conclusions. These early experiments demonstrated that a substance can be obtained from hepatic endocrine secretion, which, when injected into dogs in its purified form, causes diuresis. Thus the liver appears to have a function, not hitherto attributed to this organ, which affects urinary output. The "substance" may be hormonal in nature; that this is an endocrine function is suggested by the fact that the substance is secreted into the bloodstream and that it has a specific effect on a distant organ.

The following experiments were performed at the Department of Pharmacology, Albert Einstein College of Medicine, Yeshiva University, New York City. They were designed to study the characteristics and mode of action of the diuretic factor obtained from liver secretions. Both anesthetized and unanesthetized dogs were used.

EXPERIMENTS IN ANESTHETIZED DOGS: In an initial series of 3 experiments all dogs were anesthetized, using pentobarbital, 25 mg/kg (5 % in 20 % ethanol). The first three tests were done with the raw material obtained after lyophilization, dialyzation and re-lyophilization of the liver perfusate. (This was the same substance used for the early experiments done in Uruguay except that it was now re-lyophilized, so that it was possible to know the actual amount of material injected. The dogs were given a priming infusion of 10 cc/kg of physiologically balanced solution, containing in 220 cc a total of 88 mg of PAH and 4.5 g of creatinine. This was followed by a constant infusion of a balanced solution containing, in addition, 2.5 mg/cc of PAH and 11 mg/cc of creatinine; the solution was administered at the rate of 2.2 cc per minute. (In the third experiment of this group and in subsequent experiments, physiologic saline was administered in place of the balanced solution.) After an interval of one or two hours (allowance time), collection of urine began. It was collected every 30 minutes by means of a Malecot self-retaining catheter permanently inserted into the bladder. Midway between the beginning and the end of each 30 minute period, blood samples were taken from the jugular vein. Each time the urine was collected the bladder was immediately washed out with 15 cc of distilled water. (This additional fluid was taken into account in all calculations.) An intravenous dose of 100 mg of the liver substance suspended in 2 cc of saline solution (0.9 per cent) was administered when three consecutive periods, during which urinary flow had remained steady, were completed. The urinary flow began to increase within 30 to 40 minutes; it reached its peak within 60 to 90 minutes and did not subside completely until 3 or 4 hours later. The results were consistent in the three tests. Urinary flow increased by 100 per cent in the

first experiment and no noticeable modifications were observed in creatinine and PAH clearances (Fig. 1). In the

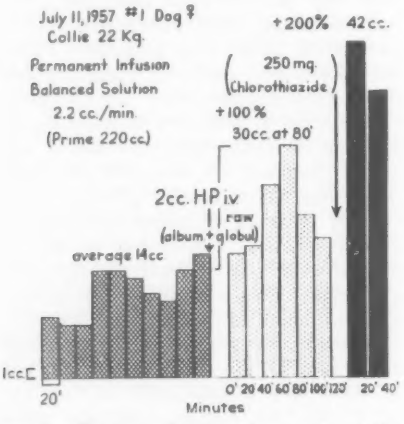

FIG. 1.—*Diagram showing the results of the first series experiments in which the "liver perfusate substance" injected was the crude material, not yet purified. The last portion of the diagram represents chlorothiazide comparative effects. In all figures, HP means Hepatic Perfusate.*

second experiment, improved techniques in the preparation of the injectable material resulted in a greater increase in urinary flow —150 per cent— while creatinine and PAH clearances remained steady. In the last experiment of this group there was a 120 per cent increase in urinary flow.

These findings again demonstrated that the substance produced by the liver increased urinary flow when administered intravenously. The clearance tests showed that it did not affect renal blood flow or glomerular filtration rate. This indicates that the mechanism of action of this diuretic factor involves renal tubular mechanism.

Purification of Liver Substance. With the completion of the first three experiments, the author was ready to proceed with the first stage of purification of the liver substance. Preliminary studies indicated that the substance was apparently protein in nature. Therefore, the material was precipitated with half-saturated ammonium sulphate in order to separate the albumin and globulin fractions. The fractions were dialyzed to remove the ammonium sulphate and then lyophilized. In order to have a basis for comparison, 100 mg was established as a standard dose of each fraction.

Experiments with Liver Substance in Stage I of Purification. The next two experiments were carried out under identical conditions: each dog received both priming and constant infusions of physiologic saline and a standard dose (100 mg) of each fraction was used. In one experiment both fractions were tested. The albumin fraction was tested first; no increase in urinary flow occurred. The globulin fraction, tested immediately afterward, produced a 20 per cent increase in urinary flow. This result was considered negative. Frequently, if more than one preparation was used in the same experiment, no

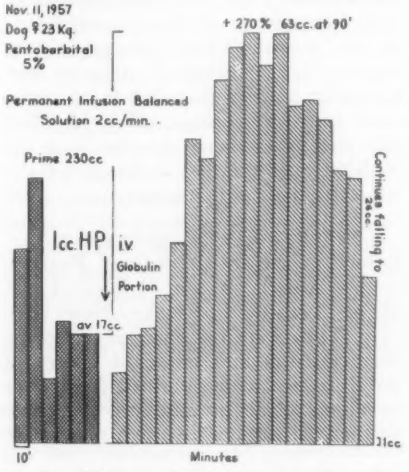

FIG. 2.—*Diagram showing the result of injection of globulin fraction of liver perfusate.*

definitive results were observed. In the other experiment, the globulin fraction alone was used. Urinary flow increased by 270 per cent (Fig. 2) (videinfra).

Second Stage of Purification. In order to achieve a further degree of purification of the liver factor, the globulin fraction, which apparently was the active fraction, was precipitated with alcohol, at various concentrations, in a cold room. This precipitate proved more easily soluble in saline solution at pH 7.4 than in water.

The substance obtained was then tested, using the usual procedure — priming dose of physiologic saline and constant infusion. A standard dose (100 mg) of 75 % —alcohol— precipitated globulin fraction was administered to a dog. An increase in urinary flow of 1000 per cent was observed (Fig. 3). For the next two tests of the globulin fraction the infusions were omitted. When a 50 mg dose of 75 % —alcohol— precipitated globulin fraction administered, a 400 per cent increase in urinary flow resulted. A standard (100 mg) dose of 20 % —alcohol— precipitated globulin fraction produced an increase in urinary flow of only 60 per cent. In the succeeding experiments described in the next section the 75 % —alcohol— precipitated globulin fraction, considered the active principle, was used. It will be referred to hereafter as Type I substance.

An experiment was performed in which the constant infusion, which regularly followed the priming dose of balanced solution, was allowed to continue for 4 hours and 30 minutes before the diuretic factor was administered. This was done to determine whether the constant infusion itself had a diuretic effect. No increase in urinary output was obtained (Fig. 4). The glo-

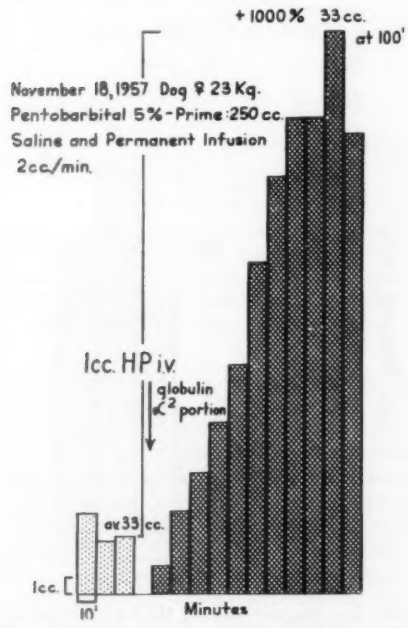

FIG. 3. — *Results of injection of purified precipitated globulin fraction referred to as Type 1 substance.*

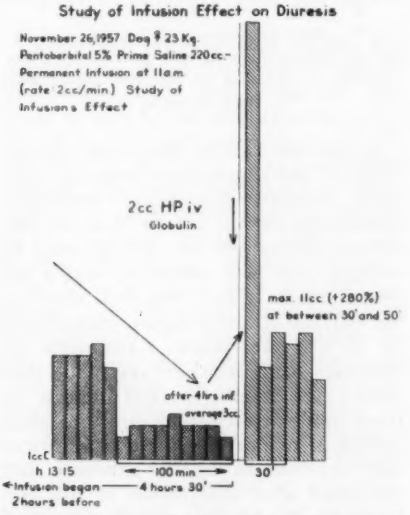

FIG. 4. — *Diagram showing that the constant saline infusion by itself does not increase diuresis.*

bulin fraction was then injected; it produced an increase in urinary flow of 280 per cent within 40 to 50 minutes. (A subsequent experiment tested the effect of continuing the constant infusion alone, without prior priming. It had no effect on urinary flow.)

Neither a priming dose of balanced solution nor a constant infusion was given in the next test. A dose of 150 mg of globulin fraction was administered and an increase in urinary flow of 1000 per cent was obtained within 110 minutes (Fig. 5).

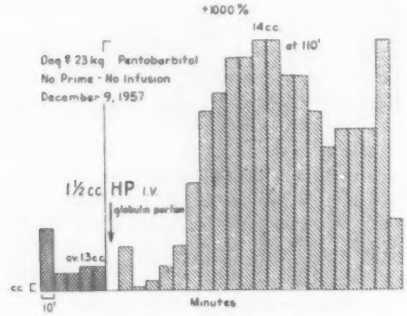

FIG. 5. — *Diagram showing that even when the dog is without priming nor permanent saline infusion, it responds to the liver factor with high diuresis.*

A test was run to determine the activity of the supernatant from the globulin alcohol precipitate. Following administration of a 4 cc dose of the supernatant, a 300 per cent increase in urinary flow was observed, which indicated that it had retained some of the marked diuretic action characteristic of the precipitate. Calculation of osmolar concentration revealed that there had been a marked increase in total solute excretion during the peak period of urinary flow. An experiment also was done to test the sediment that remained after dialyzation of the globulin fraction during the first stage of purification. Urinary flow increased by 600 per cent and osmolar concentration again revealed a marked increase in solute excretion.

EXPERIMENTS IN UNANESTHETIZED, TRAINED DOGS, WITH TYPE I SUBSTANCE: In the first experiment of this series no priming solution or constant infusion was given. A 100 mg dose of Type I solution was administered and urinary flow increased by 1360 per cent. In the second experiment the priming solution was omitted but the dog received a constant infusion. The usual dose of Type I substance was given but only a 900 per cent increase in urinary flow was obtained. In evaluating the latter result it must also be taken into account that the injection was given abruptly and that the animal was in a state of shock, with anuria, for about 15 minutes.

In the final experiments of the se-

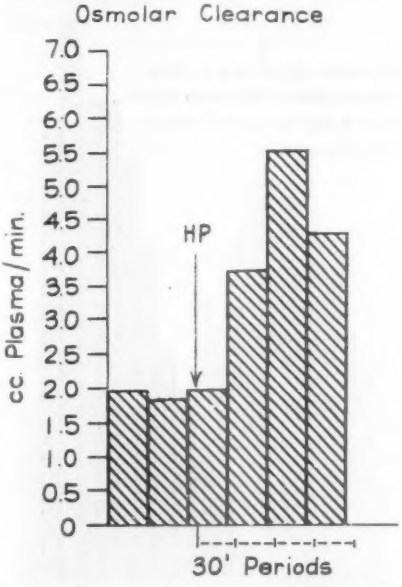

FIG. 6. — *Diagram showing the high increase in osmolar clearance, leading to a high increase in total solute excretion during the peak of diuretic effect of liver factor.*

ries, each dog received a priming solution, a constant infusion, and a 100 mg dose of Type I substance. In the third experiment urinary flow increased by 825 per cent.

A marked increase in osmolar clearance was obtained. Urine concentrations of solutes were lower, but free water clearance (hypotonic urine) was not obtained (see Fig. 9); creatinine clearance remained steady (Fig. 6).

Results in the fourth experiment were comparable to those obtained in the previous one with regard to clearance and osmolarity. Increase in urinary flow amounted to 1,120 per cent

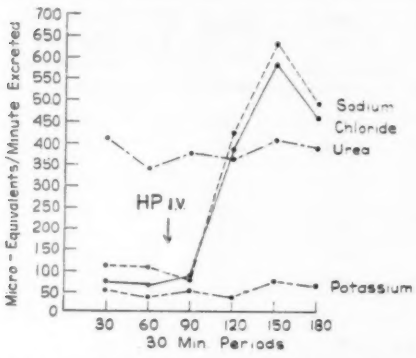

FIG. 7.—*Diagram showing the high increase in sodium and chlorides total excretion per period during the diuretic effect of the liver factor, while urea and potassium total excretion remain steady (belongs to Protocol # 1).*

The obtained free water clearance is shown in fig. 8.

In the last two tests, urine was collected every 30 minutes and solute excretion studied for each period. Sodium and chloride excretions showed pronounced increases (accounting for the pronounced increase in solute excretion), while potassium showed no significant variation. Urea excretion did not change (See Fig. 7) (See Protocols # 1 and # 2 and accompanying charts.) (Table I and Table II.)

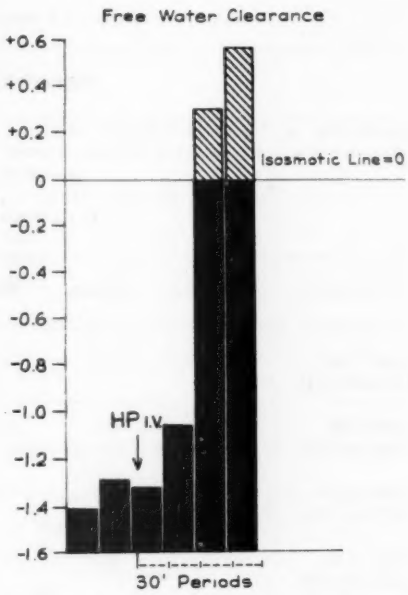

FIG. 8.—*An example of positive values of "Free water clearance" (belongs to Protocol # 1) (same results in Protocol # 2, Table 2).*

Additional Experiments. When the preceding experiments were completed, the author again considered the problem as to whether the diuretic factor found in the perfusate was a specific product of the liver. The possibility existed that it was produced in some other part of the body and was present in the blood in the liver prior to perfusion. During the infusion it could have become mixed with Tyrode's solution and, therefore, been found in the perfusate. To find the answer to this question, the author decided to divert the route by which the portal blood enters the general circulation. In a heparinized dog, the hepatic artery and inferior vena cava were ligated and the blood was carried by a cannula from the portal vein to the external jugular vein. A 40 cc sample of peripheral blood was taken from the hu-

TABLE 1.—*Example of a complete protocol (Protocol # 1).*

PROTOCOL # 1

Female dog, 21 Kg, nonanesthetized; at 10 am priming begun with 3 Gm creatinine in 50 cc saline iv; at 10:10 constant iv infusion started with 850 cc saline containing 6 Gm creatinine at rate of 2 cc/min

Urinary flow

Time	Urine	cc/min	Wash	Total	Sample
11:00-11:30	15 cc	0.5	15 cc	30 cc	# 1
11:15 BLOOD 1					
11:30-12:00	15 cc	0.5	15 cc	30 cc	# 2 at 12:05 injected 0.10 Gm Type I HP (Hepatic Perfusate) iv
11:45 BLOOD 2					
12:00-12:30	18 cc	0.6	15 cc	33 cc	# 3
12:15 BLOOD 3					
12:30- 1:00	77 cc	2.56	15 cc	92 cc	# 4
12:45 BLOOD 4					
1:00- 1:30	174 cc	5.8	15 cc	189 cc	# 5
1:15 BLOOD 5					
1:30- 2:00	142 cc	4.73	15 cc	157 cc	# 6
1:45 BLOOD 6					
2:00- 2:30	124 cc				
2:30- 3:00	70 cc		This portion was checked only for volumetric results		
3:00- 3:30	49 cc				

Osmolar clearance

Sample	mosm/l, Plasma	mosm/l, Urine	Clearance	Free water clearance
# 1	306.90	1208.8	1.903	− 1.403
# 2	301.	1092.32	1.813	− 1.313 ← HP injected
# 3	298.99	985.41	1.976	− 1.376
# 4	301.98	427.89	3.628	− 1.068
# 5	301.98	286.36	5.492	plus 0.308
# 6	305.92	274.73	4.25	plus 0.52

Creatinine clearance

Sample	mg % Plasma	mg % Urine	Clearance
# 1	22.	1650.	75.
# 2	21.	1350.	64.2
			← HP injected
# 3	21.5	1350.	68.94
# 4	21.5	420.	59.85
# 5	20.5	265.	81.43
# 6	20.5	310.	78.67

Chlorides

Sample	Plasma mEq/l	Urine mEq/l (corrected)	Micro Eq/min excreted
# 1	128.7	154.4	77.2
# 2	127.5	138.0	69.
			← HP injected
# 3	128.3	148.47	89.
# 4	128.0	151.28	388.26
# 5	128.7	101.76	590.2
# 6	115.5	95.7	453.

Potassium

# 1	3.76	112.8	56.4
# 2	3.94	73.6	36.8
			← HP injected
# 3	3.85	88.76	53.9
# 4	3.56	15.28	39.2
# 5	3.58	12.95	75.16
# 6	3.72	13.92	65.86

Sodium

# 1	165.	239.6	119.8
# 2	163.	227.	113.5
			← HP injected
# 3	157.	153.42	92.03
# 4	159.5	164.17	421.36
# 5	159.5	110.01	638.23
# 6	156.5	103.32	489.03

	Urea	
# 1	836.	416.6
# 2	688.	344.
		← HP injected
# 3	630.55	378.33
# 4	139.70	365.33
# 5	74.93	434.6
# 6	82.87	392.23

meral vein before the experiment and another 40 cc sample was collected one hour and 15 minutes after.

Both blood samples were processed in the usual manner and the results revealed that while each of the samples contained the diuretic substance, the second sample contained 22 mg less than the first. In other words, when not secreted by the liver, the substance was found in a reduced amount in the peripheral blood. Although this might appear to be an answer to the question, the author rejected it as such because elaboration of normal plasma proteins is a function of the liver and it could be objected that the exclusion of the liver could be responsible for the lower

TABLE 2. — *Protocol # 2 showing that results are consistent.*

PROTOCOL # 2

Female dog, 21 Kg, nonanesthetized; at 10:15 am priming with 50 cc saline containing 3 Gm creatinine; at 10:30 am started constant iv infusion with saline 850 cc containing 6 Gm creatinine at rate of 2 cc/min

	Urinary flow				
Time	Urine	cc/min	Wash	Total	Sample
11:30-12:00	22 cc	0.73	15 cc	37 cc	# 1
11:45 BLOOD 1					
12:00-12:30	18 cc	0.6	15 cc	33 cc	# 2 at 12:35 injected 0.10 Gm Type I HP (Hepatic Perfusate) iv
12:15 BLOOD 2					
12:30- 1:00	20 cc	0.66	15 cc	35 cc	# 3
12:45 BLOOD 3					
1:00- 1:30	76 cc	2.53	15 cc	91 cc	# 4
1:15 BLOOD 4					
1:30- 2:00	171 cc	5.7	15 cc	186 cc	# 5
1:45 BLOOD 5					
2:00- 2.30	161 cc	5.36	15 cc	176 cc	# 6
2:30- 3:00	107 cc	3.56	15 cc	122 cc	# 7 (only volumetric)

Osmolar clearance

Sample	mosm/l Plasma	mosm/l Urine	Clearance	Free water clearance
# 1	300.98	901.93	2.187	− 1.457
# 2	295.08	983.48	2.	− 1.4
				← HP injected
# 3	300.	821.56	1.808	− 1.148
# 4	294.09	438.11	3.768	− 1.238
# 5	298.03	278.90	5.33	plus 0.37
# 6	300.98	260.57	4.614	plus 0.746

Creatinine clearance

Sample	mg % Plasma	mg % Urine	Clearance
# 1	22.	2182.	72.40
# 2	22.	2383.	65.
			← HP injected
# 3	23.	2100.	60.26
# 4	22.	502.8	57.82
# 5	21.	271.9	78.56
# 6	20.	278.7	74.70

Chlorides

Sample	Plasma mEq/l	Urine mEq/l (corrected)	Micro Eq/min excreted
# 1	116.1	192.47	141.14
# 2	117.2	230.96	138.37
			← HP injected
# 3	117.2	179.2	119.46
# 4	115.7	147.35	373.28
# 5	116.5	101.52	578.66
# 6	113.5	87.11	467.49

Potassium

# 1	3.57	73.29	53.75
# 2	4.05	47.11	28.26
			← HP injected
# 3	4.23	78.4	52.27
# 4	3.54	13.58	34.41
# 5	3.69	9.35	53.29
# 6	3.93	11.26	60.42

Sodium

# 1	149.3	182.05	132.90
# 2	147.7	207.68	124.61
			← HP injected
# 3	148.1	149.45	98.64
# 4	147.7	167.82	424.59
# 5	151.2	110.55	630.13
# 6	149.8	115.42	410.90

Urea

# 1	509.34	373.51
# 2	601.22	360.73
		← HP injected
# 3	463.75	309.16
# 4	126.52	320.52
# 5	67.18	382.92
# 6	70.50	378.35

amount of proteins in the plasma, even of no specific diuretic proteins were produced. Therefore the author attempted another approach. The liver was perfused with Tyrode's solution in the customary manner except that each of the 7 liters used for the perfusion was collected and processed separately so that by the time the seventh liter passed through the liver, all traces of blood had been washed away by the first six liters. A constant infusion was given to an unanesthetized dog at the rate of 2 cc min. The infusion was continued for 1 hour and 20 minutes until a steady rhythm of urinary flow had been established. At this time the dog received 45 mg of Type I substance obtained by precipitation of the blood-free liver perfusate (Liter # 7). Diuresis began 25 minutes after injection was given and an increase in urinary flow of 1000 per cent was obtained. Thus the substance recovered from the blood-free seventh liter proved exceedingly active. This experiment indicated again that the diuretic factor that can be found in the peripheral blood is a product of the liver and that it is secreted into the bloodstream by that organ.

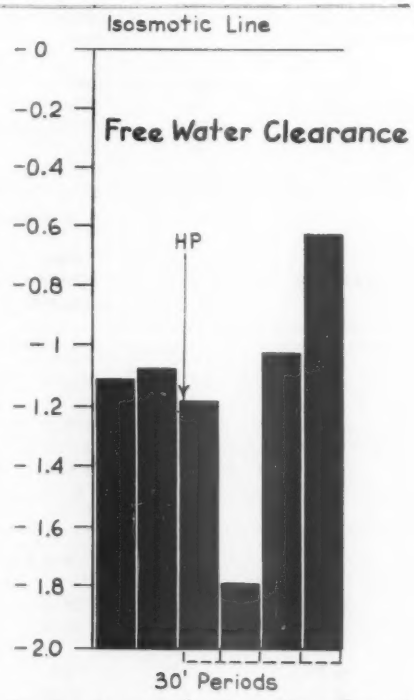

FIG. 9.—*An example of negative values of "Free water clearance", but with a tendency towards positive values, same direction as shown in fig. 8.*

A second question arose when it was suggested that, according to Brandt et al. [22], the diuretic action of the liver factor might be attributed to contamination by pyrogens. The results of experiments, during which rectal temperature checks were maintained, demonstrated that the diuretic and natriuretic action of the liver product are not linked in any way to pyrogenic activity. A permanent infusion with saline solution was performed at the rate of 2 cc/min. Rectal temperature at this time was 103°F. Three hours later, when the dog received a 50 mg dose of Type I substance obtained by precipitation of Liter # 2 perfusate, rectal temperature was 101°F. An increase of 800 per cent in urinary flow was obtained and the temperature at the time of peak diuresis was 101.8°F.

It should be pointed out that most of the diuretics presently employed in clinical medicine are probably contaminated to some extent by pyrogens. Certainly much of the material used in tests of renal physiology is not free of pyrogenic contaminants; it would be extremely difficult to eliminate them entirely. More important, Lathem [23] has reported recently that the pyrogenic reaction in man does not provoke increased urinary excretion of water and sodium. But irrespective of these considerations, the author wishes to emphasize that, in the case of the present study, the results of experiments during which a temperature check was maintained demonstrated that the diuretic action of the liver substance is not associated with pyrogenic activity.

Discussion

Dr. Homer W. Smith's recent outstanding paper on "Salt and Water Volume Receptors" [24] has been used as a guide for this discussion, which is an effort to correlate the information obtained about the liver substance with what is known, and as yet unknown, on renal physiology.

For example, Smith makes reference (p. 628) to the findings of Blomhert et al. [14] that parenteral administration of saline solution at night does not provoke diuresis, while oral administration does. According to Smith this represents an unsolved problem. Perhaps the presence of a hepatic diuretic factor provides an explanation. In the author's experiments, instead of water being orally administered, the diuretic factor, which normally would be liberated at the time orally ingested water passed through the liver, is parenterally administered.

Smith also refers to the fact (p. 631) that constriction of the vena cava under the diaphragm results in sodium retention. Such a procedure would prevent the hepatic substance from entering the general circulation from the hepatic vein and so prevent it from exerting its natriuretic effect.

The result of Ladd's [13] experiment, which demonstrated that water taken orally prior to intravenous administration of saline solution results in a significant diuresis, which could not be obtained with the intravenous infusion alone, could be accounted for by the liberation by the liver of the diuretic substance. In the present experiments, the previous oral ingestion of water was replaced by administering the liver substance, and thus the same diuretic result was obtained.

On page 634 Smith writes "...no physiologic circumstance is known which leads to... unequivocal natriuresis..." The present experiments suggest that this physiologic circumstance exists. Oral ingestion of water, because it passes through the liver, may liberate the natriuretic factor when there is an excess of sodium in the body.

With regard to experiments that demonstrate that the diuretic response

may vary with the position of the body, the author points out that in all the tests made during this study, the position of the body (supine) was the same before and after injection of the liver factor. However, experiments performed by Von Arman [25] in Chicago with the author's liver factor (which, in essence, confirmed the author's findings) were done in trained, unanesthetized, catheterized dogs, which were kept in a standing position during the entire experiment. In other words, the results are the same whether a supine or a standing position is used.

Another point of interest is the phenomenon described by Pappenheimer and Kinter, [26-28] the post-glomerular separation of cells and plasma in the renal circulation with its possible effect on urinary excretion. The experiments with the liver substance demonstrated that there were no alterations in blood pressure, renal plasma flow, or glomerular filtration rate when the substance was administered. Therefore it is not likely to alter the phenomenon involved in the Pappenheimer and Kinter theory.

In view of the results described, the author feels that when considering the antidiuretic and antinatriuretic systems presently known, a diuretic and natriuretic system involving the liver must also be taken into account as forming part of the "integrative systems" that serve homeostasis. Further experiments will be carried out to determine whether any relationship exists between this liver function and that of the hormones of the adrenal cortex and neurohypophysial system.

Summary and Conclusions

This report covers research which has been carried out to determine whether or not the liver plays a role in causing the "delay time" before diuresis to be of shorter duration when water is taken orally than when parenterally administered. Perfusion of the liver was performed in dogs and the perfusate tested for diuretic effect. Results obtained in both anesthetized and unanesthetized animals revealed increases in urinary flow of up to 1300 per cent.

A study of the mode of action of this new hepato-diuretic factor revealed that both PAH and creatinine clearances remained steady, demonstrating that no modifications had occurred in either renal plasma flow or glomerular filtration rate. These findings indicated that the mechanism of action of this diuretic factor involves renal tubular mechanisms. The study (Fiske Osmometer) of osmolar clearance revealed pronounced increases in total solute excretion and at the same time, because large quantities of water were excreted, dilute urine sometimes was obtained almost to the point of free water. Total urea and potassium excretion remained unchanged but the curve of the increase in sodium and chloride excretion was about parallel to that of total solute excretion.

It seems evident, therefore, that this is a diuretic-natriuretic substance that acts directly or indirectly on the renal tubules and that it is produced by the liver, presumably as an endocrine secretion. Further studies are in progress related to purification, isolation, and identification of this factor, which is apparently of a protein nature or is bound to a protein. Studies are in progress also to determine whether the substance acts directly or functions in relation to other systems, such as those of the adrenal cortex or the neurohypophysis.

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PROCEEDINGS OF THE ARGENTINE SOCIETY
OF BIOLOGY

(BUENOS AIRES, ARGENTINA)

October 1, 1959

Thyroid and resistance to cold. C. M. GARRIDO. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires. Comisión Nacional de Energía Atómica, Buenos Aires).*

1) The time of death of albino rats, iodothyroidectomized since 20 days before, submitted to intense cold was shorter than that of intact rats used as controls;

2) The time of death of iodothyroidectomized albino rats submitted to intense cold, was longer when the animals were given levothyroxin (6 μg) and tri-iodothyronine (2 μg);

3) Larger doses (10 γ) of tri-iodothyronine had a less marked protective effect;

4) Prednisone has a slight protective effect in iodothyroidectomized animals, submitted to the action of a very intense cold.

Iodothyroidectomy in the toad. J. C. TRIVELLONI, A. O. DONOSO AND G. E. BUR. *(Instituto de Fisiología, Facultad de Ciencias Médicas, Buenos Aires, Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

A technique of thyroidectomy in the adult toad, with radioactive iodine (I^{131}) and the progressive lesions up to total destruction of the gland, was described.

Sixty days after injection of radioactive iodine, residual tissue or regeneration was not observed.

Biochemical and histochemical study of fluorescent serum protein distribution in the connective tissue. R. E. MANCINI, O. VILAR, C. GÓMEZ, J. M. DELLACHA, O. W. DAVIDSON AND A. CASTRO. *(Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Buenos Aires, Argentina).*

The rat serum proteins were bound "in vitro" with a fluorescent dye —Lissamine Rhodamine B 200— using the Chadwick's method with slight modifications. This serum was studied by electrophoresis and the amount of protein bound dye was determined. The absorption curves in the visible and ultraviolet spectrum of the labelled and unlabelled proteins were also determined.

The labelled serum was injected i.v. to albino rats weighing 150 to 200 g, the dosis were between 0.6 to 1.2 cm^3 per 100 g of body weight. Three animals were killed at several periods between 10 minutes and 12 days after injection. Blood was obtained before death to study the dye concentration in the serum, which was studied electrophoretically. After death several tissues were fixed and the slides were seen in the ultraviolet microscope.

It is observed: 1) the dye is bound predominantly to albumin; 2) the concentration of labelled protein, as measured by the amount of dye bound to it, diminishes to one third in 12 hours; then the diminution is slower and the labelled protein disappears from the circulation on the 16th day after injection; 3) the labelled protein appears in the vas-

cular lumen, capillary walls, Kupffer cells, venous sinuses of the spleen and lymphatic sinuses of the hemolymphatic nodes 30 minutes after injection. The fluorescent protein also is accumulated early in the ovarian and thyroid follicles. Twelve hours later fluorescence is seen in the cutaneous and submucous connective tissues, fibrous septa and capsule, interstitial tissues, and basal membranes of many organs.

At 24 hours it almost disappears from the basal membranal and connective tissue; it diminishes in the vascular lumen and appears as small droplets in the cells of the tubuli contorti of the kidney.

All these findings indicate that there is an equilibrium among the serum proteins, the connective tissue (as temporary place for storage of them) and the R. E. S., thyroid, ovary and renal tubuli contorti cells (a place for prolonged deposition of the serum proteins).

Diabetogenic and antidiabetogenic action of triamcinolone and dexamethasone. J. M. DE CORRAL SALETA, J. C. PENHOS AND A. F. CARDEZA. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

1) The effect of triamcinolone and dexamethasone on the blood sugar level of dogs, rats and toads with pancreatic mass surgically reduced was studied; experiments were also done in normal toads.

2) Both triamcinolone and dexamethasone had an intense transitory diabetogenic action in dogs (a permanent effect was not searched) the action of dexamethasone being more active.

3) This diabetogenic action was more intense with both triamcinolone and dexamethasone than with the corticoids previously used by other investigators.

4) In rats with large partial pancreatectomy and prolonged treatment, low doses (10 and 50 μg) of triamcinolone and 10 μg of dexamethasone showed a preventive effect on diabetes in a certain number of animals: 45 %; 62 % and 84 % respectively.

5) Triamcinolone at higher doses (250 μg) had a diphasic action: an initial diabetogenic and, later, a protective one. Dexamethasone at doses of 50 and 250 μg showed to be toxic.

6) The diabetogenic action of both substances was also observed in toads, if the pancreas was surgically reduced and even if pancreatectomy was simultaneous to hypophysectomy but not in normal animals.

Normal hematological values in young residents in Mendoza (747 m above sea level). EGLANTINA Y. SOTTANO, F. O. FERNÁNDEZ, E. O. ZANGHERI AND J. R. E. SUÁREZ. *(Instituto de Cardiología y Cátedra de Fisiología, Facultad de Ciencias Médicas, Mendoza).*

The study of series of normal men and women, residents in Mendoza (747 m over the sea level) shows similar values to those living in the plain. The few exceptions with significant differences observed in some isolated series, are not enough to alter this picture, specially when considering the smaller number of cases in the series of women.

November 5, 1959

Hemoglobin study in residents at 4515 m above sea level. G. RATHE. *(Instituto de Biología de la Altura, Universidad Nacional de Tucumán, S. S. de Jujuy, Argentina).*

No statistically significant differences were found between the hemoglobin of men living from long time at 4515 meters and at 1270 meters of altitude. The methods used were paper electrophoresis and denaturation by alkali in one minute.

Action of orinase on the glycemia of the turtle "Phrynops hilarii". MARIA MARQUES AND P. RIET CORRÊA. *(Instituto de Fisiología Experimental, Facultad de Medicina, Porto Alegre, Brasil).*

Orinase, in doses of 500, 250, 150 and 50 mg/Kg administered respectively by mouth, intraperitoneally, subcutaneously and intravenously, produced hyperglycemia in the turtle *"Phrynops hilarii"*, this effect being statistically significant only at the doses of 500 mg/Kg.

Control groups treated with distilled water or with the Orinase diluent (NaOH 0.1 N),

also exhibited hyperglycemia, but this was more moderate and lasted for a shorter time than with Orinase.

Turtles treated with 200 mg/Kg of Orinase by mouth did not show changes in the blood sugar level, but the controls had an increase in their blood sugar levels. The difference between the two groups is statistically significant.

Orinase diminished the hyperglycemic effect caused by adrenaline, but had no influence upon the glucagon effect at the doses used.

Action of stilboestrol on DPNH-oxidase and succinoxidase. A. O. M. Stoppani, J. A. Brignone and C. C. de Brignone. *(Instituto de Química Biológica, Facultad de Ciencias Médicas, Universidad de Buenos Aires).*

The actions of stilboestrol (4-4'-dihydroxidiethylstilbene) on heart muscle succinoxidase and DPNH-oxidase systems have been studied comparatively. The DPNH-oxidase is far more sensitive than the succinoxidase system to stilboestrol which inhibits DPNH oxidation at 10^{-6} M concentration. The inhibition is not reversible and seems to take place between cytocromes b and c. Stilboestrol does not affect succinic dehydrogenase or cytochrome oxidase but prevens the oxidation of exogenous cytochrome c by the later enzyme. Oxidation of succinoxidase by BAL is not affected by stilboestrol.

Adaptation of epidemic hemorrhagic fever virus. Norma E. Mettler. *(Cátedra de Microbiología y Parasitología de la Facultad de Ciencias Médicas de Buenos Aires).*

Adaptation of hemorrhagic epidemic fever virus was obtained by intraamniotic innoculation of 7 days old chick embryos. In the first passage we observed pocks on the C.A.M. Further passages on 11 days old chick embryos C.A.M. gave typical lesions.

Action of histaminase (diamino-oxidase) on the gastric secretion by histamine in the dog. B. B. Lozzio and M. Royer. *(Instituto de Gastroenterología, Instituto Nacional de la Salud, Ramos Mejía, Buenos Aires).*

Intravenous injection of histamine diminished markedly the gastric secretion of hydrochloric acid and pepsine produced by histamine. In 8 dogs, 30 μg of histamine per Kg of body weight were injected subcutaneously. In 7 dogs 12 μg of histamine per Kg of body weight were injected 20 minutes after the histaminase.

Contribution to the study of the basal membrane of human seminal tubules. M. H. Burgos. *(Instituto de Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza).*

The basal membrane of the human testis is formed by an elastic lamellar structure embedded in a mucoid material. The basal membrane sends into the tubular wall numerous projections.

It is suggested and discussed the meaning of this projections as a way for cellular fixation and metabolic interchange.

There was no evidence of smooth muscle or mioepithelial cells in the basal membrane or tunica propia.

(Córdoba, Argentina)
June 11, 1959

Influence of sexual hormones in the concentration of oxytocin in the neurohypophysis. R. P. Deis. *(Instituto de Investigación Médica "Mercedes y Martín Ferreyra", Casilla de Correo 389, Córdoba, República Argentina).*

The concentration of oxytocin in the neurohypophysis in rats and the influence of sexual hormones were studied.

The concentration of the hormone in female rats was of 451 ± 31 mU and in male rats of 359 ± 16 per 100 g of body weight; the differences between both sexes were statistically significant. Castration determined an increase in the concentration of the hormone in both sexes. The injection of testosterone 500 μg per day for 7 days into spayed female rats or the injection of Estradiol (50 μg per day for 7 days) into castrated male rats diminished the values to figures similar to those obtained in normal animals.

The injection of Progesterone (500 μg per day for 7 days) into normal female rats did not modify the oxytocin value in the neurohypophysis.

Influence of neurohypophyseal hormones on the water excretion through the toad's kidney. S. TALESNIK AND E. CAPMOURTERES. *(Instituto de Investigación Médica "Mercedes y Martín Ferreyra", Casilla de Correo 389, Córdoba, Argentina).*

Diuresis and inulin clearance was studied in the toad *Bufo arenarum* Hensel. Injection of vasopressin produced a diminution of glomerular filtration rate and increased tubular reabsorption of water. A 30 mU dose of oxytocin caused an increase in glomerular filtration rate and in tubular reabsorption of water.

The activity of vasopressin in changing renal function was 8 times greater than that of oxytocin.

Injection of hypertonic saline solution produced a modification of renal function which was similar to that produced by vasopressin and this action could be abolished by hypophysectomy. The renal response to dehydration was not modified by hypophysectomy.

(BUENOS AIRES, ARGENTINA)
April 7, 1960

Erythropoietic activity of urinary extract of high altitude residents. J. L. SCARO. *(Instituto de Biología de la Altura, Universidad Nacional de Tucumán, S. S. de Jujuy, Argentina).*

The urine alcohol extracts of permanent residents in Jujuy at 1260 m above sea level, does not show erythropoietic activity when injected to the rat.

The urine extracts of new comers at high altitude show an erythropoietic activity whose value is highly significant 48 hours after arriving at 3990 m.

The weight of the alcohol extract obtained from 1000 cm^3 of urine in the recently arrived residents increases proportionally with their erythropoietic activity.

Some histological and histochemical modifications of adrenal cortex in rats under intense cold. C. M. GARRIDO. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

The histological and histochemical study of adrenals of white rats under the intense action of cold showed:

1) dissappearance of differential zone between the fascicular and reticular layer and dilation of sinusoids in the fasciculated zone;

2) intense depletion of lipids two hours after, and almost total depletion in rats dead by the intense action of cold;

3) diminution of ascorbic acid two hours after, and almost total disappearance of the adrenals in rats dead by the action of cold;

4) slight modifications in alkaline phosphatase.

Influence of ph and toxics on imbibition and release of spermatozoa in the testicle of the toad. J. J. ASTRADA. *(Instituto de Investigación Médica "Mercedes y Martín Ferreyra", Casilla de Correo 389, Córdoba).*

The effect that variations of pH, as well as that of toxic drugs known to inhibit enzimatic actions, exert upon imbibition and spermatozoa release of toads' testis *in vitro*, was studied.

1) When pH ranges above 3 or below 11, the process is normal. At pH 3 and 11, the testis' habitual release of spermatozoa in hypotonic medium is inhibited. The phenomenon is reversible. When pH is below 3 or above 11, the testis are damaged.

2) Potassium cyanide reversibly inhibits the releasing process, even though the testis be sufficiently stimulated by hydration.

3) Addition of hypophysis produces hydration and spermatozoa release. Sodium chloride seems to facilitate the release.

Salivary glands in hypophysectomized dogs. J. J. ARGONZ. *(Instituto de Biología y Medicina Experimental, Obligado 2490, Buenos Aires).*

Submaxillary glands of hypophysectomized dogs were studied and the results were compared with those obtained from normal controls. It was found a diminution of: a) submaxillary gland weight; b) quantity of salive

resulting from timpanic cord electrical stimulation or pilocarpin; c) diameter of tubules and acini.

Histophysiological observations on the reproduction of "ciona intestinalis". R. NARBAITZ. *(Instituto de Anatomía General y Embriología, Facultad de Ciencias Médicas, Buenos Aires).*

The present study was made in order to know if auto-sterility and homo-fertility in *Ciona*, can be related to the existence of diffusible substances in the eggs, which could modify the behavior of spermatozoa.

Our experiments failed to demonstrate the presence of these substances, using techniques similar to those used by other authors in the study of fertilisin in eggs of echinoderms.

The observation with the phase contrast microscope of the behavior of spermatozoa in the presence of eggs denudated by various procedures shows that the recognition of gametes occurs after their contact is established.

In confirmation of Morgan's works, we obtained self-fertilization through the use of an acid medium.

The histological study of both gonads showed several details of special interest: a) The seminiferous tubules are included in the intestinal wall. b) Their organization resembles the one of higher vertebrates. c) The follicular cells of the eggs seem to derive from connective cells surrounding the young oogonia, and not from degenerated ova as has been stated. d) The peri-ovular space does not contain mucopolysaccharides, and thus is not equivalent to the jelly layer of echinoderm eggs. The electron microscopy of the contact between gametes and the biochemical study of lysins seem to be the logical steps to follow in the study of this problem.

May 5, 1960

"In vitro" action of amphotericin B on the "Trypanosama cruzi". H. ABITBOL, R. E. PATTINI AND J. SALVADOR. *(Cátedra de Farmacología, Departamento de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza).*

The action of an antibiotic, Amphotericin B on *Trypanosoma Cruzi* has been studied.

Amphotericin B which is used in the treatment of "micosis", is also effective on *Trypanosoma Cruzi* (destruction or lisis) *in vitro* at similar doses used by W. Gold et al.

Effectiveness of the same antibiotic *in vivo* is being presently investigated.

Action of some synthetic gestagens on the evolution of pregnancy in the rat. E. MONTUORI, G. E. BUR AND A. KOROMPAY. *(Departamento de Investigaciones de los Laboratorios Dr. Gador y Cía. y Laboratorio de Patología del Hospital Rivadavia, Buenos Aires).*

All the steroids employed, when they are administered from the first day, affect the development of pregnancy. Testosterone and the 19-nortestosterone compounds in doses of 1 mg inhibit the implantation in 100 % of the animals, while with progesterone, acetoxyprogesterone and its 6-methyl-ester, the number of viable animals on term per litter is decreased. These three last steroids do not affect the development of pregnancy if they are administered from the 14th day, the contrary to what occurs with testosterone and the 19-norderivatives.

Action of some synthetic gestagens on the ova segmentation in the rat. G. E. BUR, E. MONTUORI AND A. KOROMPAY. *(Departamento de Investigaciones de los Laboratorios Dr. Gador y Cía. y Laboratorio de Patología del Hospital Rivadavia, Buenos Aires).*

These preliminary studies showed that the steroids used exert an action since the earliest periods of ovular segmentation.

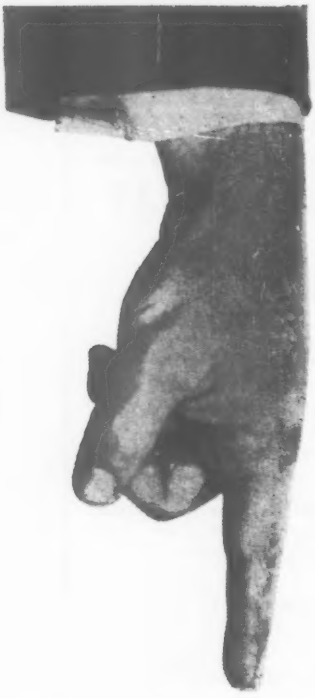
Study of radiactive sodium distribution in the normal rat. S. A. D'AGOSTINO. *(Instituto de Biología y Medicina Experimental, Obligado 2490 y Comisión Nacional de Energía Atómica, Buenos Aires).*

1) The modification of the sodium space in function of time was studied. The state of

equilibria was observed at the end of the first hour.

2) The sodium and thiocyanate spaces in relation to weight were studied. Their regression coefficients were not statistically different. The sodium and thiocyanate spaces measured one hour after the injection were 26.93 ± 0.33 ml/100 g and 35.86 ± 1.61 ml/100 g respectively.

3) The distribution of Na^{24} in striated muscle, spleen, liver and kidney in relation to the plasma activity was determined.



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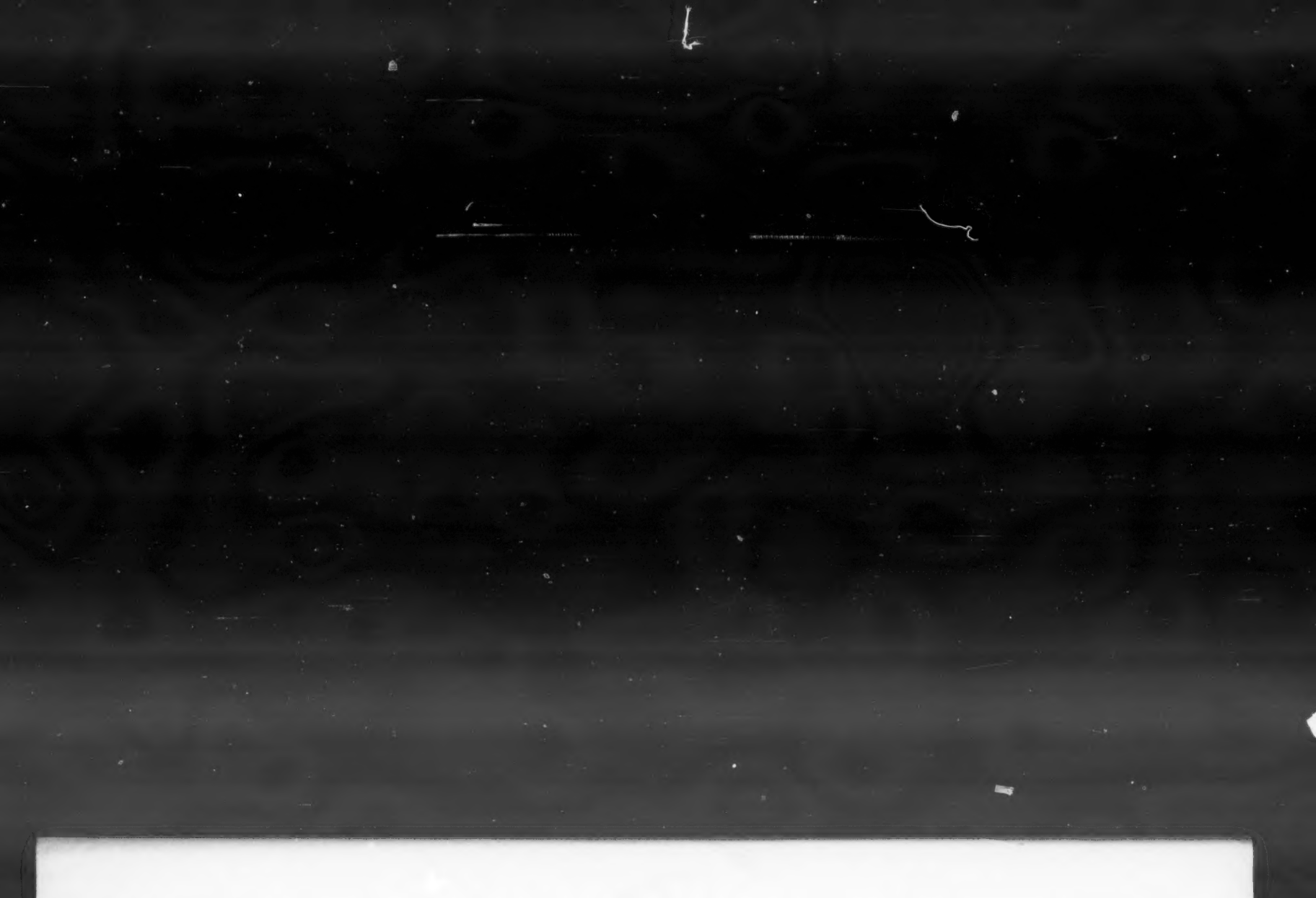

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(2) WHITTEMBURY, G., RAMÍREZ, M., FERNÁNDEZ, J., MONGE, C.: *Acta physiol. lat.-amer.*, 1955, *5*, 117.

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metro	m	m	litro	l	l
centímetro	cm	cm	centímetro cúbico	cm^3	cc
milímetro	mm	mm	mililitro	ml	ml
micrón	μ	μ	kilogramo	kg	kg
milimicrón	$m\mu$	$m\mu$	gramo	g	gm
Angström	Å	Å	miligramo	mg	mg
microgramo	μg	μg	miliequivalente	mEq	mEq
hora	h	hr	Curie	c	c
minuto	m	Min	Milicurie	mc	mc
segundo	s	sec	Microcurie	μC	μC
milisegundo	ms	msec	por ciento	%	%

Para evitar la confusión derivada de la notación decimal diferente según los países, se adopta el punto decimal y se suprime toda notación entre millares sustituyéndose por un espacio: 10 000 (no 10.000 ni 10,000) —0.90 (no 0,90).

Corrección de pruebas: Una de las pruebas de imprenta será remitida a los autores, quienes deberán devolverlas corregidas, dentro de las 48 horas subsiguientes a su recepción. **Las modificaciones fundamentales en la corrección de las pruebas en desacuerdo con los originales no serán tomadas en cuenta.**

Pedido de apartados: Los autores que desean apartados deben solicitarlos al devolver las pruebas de imprenta. El costo de los mismos correrá por cuenta del autor.